UCL EASTMAN DENTAL INSTITUTE

ORAL MEDICINE UNIT

The LEONIDAS-2 study

Long-term Evaluation of the effectiveness Of a Novel Intra-oral electro-stimulator for the treatment of raDiotherapy-ASsociated xerostomia

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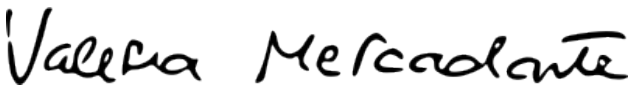
October 2015

The current project is a UCL "Grand Challenge 100" PhD training programme, funded by the UCL School of Life and Medical Sciences and the National Institute for Health Research (NIHR) Biomedical Research Centres at UCLH, Moorfields and Great Ormond Street Hospitals.

Declaration

I, Valeria Mercadante, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.



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Abstract

Head and neck cancer is the 7th most common cancer worldwide and the 7th leading cause of cancer-related death. The vast majority of head and neck cancers are diagnosed at advanced stage, which is characterised by large volume disease and locoregional metastases. The overall 5-year survival rate of head and neck cancer is 50%, although most patients with advanced stage cancers die within few years from diagnosis because of relapsing incurable disease.

Head and neck cancer patients are often managed with radiotherapy, either as main treatment modality or in association with chemotherapy and surgery. Radiotherapy can cause acute and long-term complication, of which chronic xerostomia is one of the most debilitating.

Current treatment of radiotherapy-associated xerostomia encompasses systemic and local therapeutic strategies. None of them, however, represent the ideal treatment in terms of efficacy and safety.

Neuro-stimulation of salivary glands via electrical stimulation was initially introduced 30 years ago. It is suggested to provide the benefit of increasing natural salivation with no relevant adverse side effects.

Studies using the first generation of electrostimulating devices suggested clinical significant benefit in a small cohort of individuals with radiotherapy-associated xerostomia. More recently, the use of a second-generation intra-oral device, as well as facial transcutaneous nerve stimulation, has been associated with an increase in saliva production and reduction in xerostomia symptoms.

Although promising, current data do not provide robust evidence and highlight the need of further and better designed clinical trials in order to investigate the real benefit of salivary electrostimulation in the post-radiotherapy setting.

We have performed a double-blind randomised clinical trial (The LEONIDAS-2 study) to assess the long-term efficacy of the second generation intraoral electrostimulating device in lessening xerostomia symptoms, increasing salivary gland function and improving quality of life in individuals with radiotherapy-associated xerostomia. The clinical trial took place between January 2012 and January 2015 at the University College London Hospital and Bradford Royal Infirmary (UK). Eighty-four participants were randomised to use an active electrostimulating device (providing mechanical and electrical stimulation) or a sham device (providing mechanical but not electrical stimulation) for 12 months. Randomisation was by computer-generation and analyses were performed on an intention-to-treat basis. Sixty-eight participants completed the trial. At 12 months an improvement in xerostomia symptoms (VAS) compared to baseline was observed, with no significant difference in means between the active and sham group. Salivary flow rate, measured through sialometry, was higher in the active group but the difference between the two groups was not statistically significant. At 12 months there was an improvement in quality of life, with no significant difference between the active and the control groups.

The LEONIDAS-2 clinical trial showed that salivary electrostimulation through the second generation intra-oral electrostimulating device is safe but not more effective than mechanical stimulation in relieving dry mouth symptoms,

increasing salivary function or improving quality of life in patients with radiotherapy-associated xerostomia.

Acknowledgements

I would like to thank my PhD primary supervisor, Dr Stefano Fedele, for his inspirational guidance and support. He has set an example of excellence as a researcher, mentor and role model.

I am hugely indebted to Professor Stephen Porter, secondary supervisor, for showing great interest in my research and for giving me precious advice regarding the topic of my research.

I acknowledge the UCL SLMS Grand Challenge Studentships for funding my PhD, providing a highly-supportive environment.

I would like to thank the Head and Neck Cancer Teams at the Royal Marsden (Professor Nutting and Professor Harrington) and at University College of London Hospital (Dr Carnell and Dr Mendes). Everyone made me feel very welcome during the recruitment phase of the clinical trial.

I am grateful to the staff of the Eastman Clinical Investigation Centre for the help provided and to my dear friends and colleagues at the Eastman Dental Institute, particularly Elena, Arwa, Polly and Marco.

I would like to thank Dr. Rachel Leeson, for encouraging me with her kind words and smiles.

On a more personal level, a special thank goes to my future husband, Alessandro, who generously and constantly provided moral support, encouragement and the most delicious food. I admire him enormously.

Finally, I want to express my appreciation to my parents and my brother for their love, endless support, and for never failing faith in me.

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List of abbreviations

2DCRT	Two dimensional conventional radiotherapy
3D-CRT	Three dimensional conformal radiotherapy
ASR	Age-standardized rates
CCND1	Protein cyclin D1
CDKN2A	Cyclin-Dependent Kinase Inhibitor 2A
CHART	Continuous Hyperfractionated Accelerated Radiotherapy
CRT	Chemoradiotherapy
DAHANCA	Danish Head and Neck Cancer Study Group
EBV	Epstein-Barr Virus
EGFR	Epidermal growth factor receptor
EORTC	European Organization for Research and Treatment of Cancer
FDA	Food and Drug Administration
Gy	Gray
HBOT	Hyperbaric oxygen therapy
HNC	Head and neck cancer
HNSCC	Head and Neck squamous cell carcinoma
HPV	Human Papilloma Virus
HR	Hazard ratio
IMRT	Intensity modulated radiotherapy
LRC	Local-regional control
OR	odds ratio
ORN	Osteoradionecrosis
PI3Ks	phosphoinositide 3-kinases
PTEN	phosphatase and tensin homolog
QoL	Quality of Life
RCT	Randomised clinical trial
RT	Radiotherapy
RTOG	Radiation Therapy Oncology Group
SMGs	Submandibular glands
VAS	Visual Analogue Scale
XI	Xerostomia Inventory

1 Introduction: Xerostomia in head and neck cancer survivors

1.1 Head and Neck Cancer

1.1.1 Definition and Epidemiology

The term head and neck cancer (HNC) defines the group of malignancy that can arise in the oral cavity, pharynx and larynx (including the paranasal sinuses and the salivary glands) (Mehanna et al., 2010).

The overwhelming majority of malignant tumours arising from the mucosal surfaces of the upper aerodigestive tract are squamous cell carcinomas in nature (HNSCC) (Cooper et al., 2009).

Worldwide HNC is the seventh most common cancer with more than half million cases occurring per year. These figures encompass 300,373 cases of lip and oral cavity cancers, 229,078 pharyngeal and 156,877 laryngeal cancers (Ferlay et al., 2012). Head and neck cancer is also the 7th leading cause of cancer-related death with global deaths projected to rise from 375,000 in 2013 to 575,000 in 2030 (Ferlay et al., 2012).

Figure 1-1 shows the distribution of HNC by site in England and Wales as reported by the National Head and Neck Cancer Audit (DAHNO, 2014). The audit included in the analysis 8,429 patients, a number which is close to the 9696 estimated new cancers in UK (Ferlay et al., 2012). In the graph, only malignant tumours arising in the salivary glands have been included.

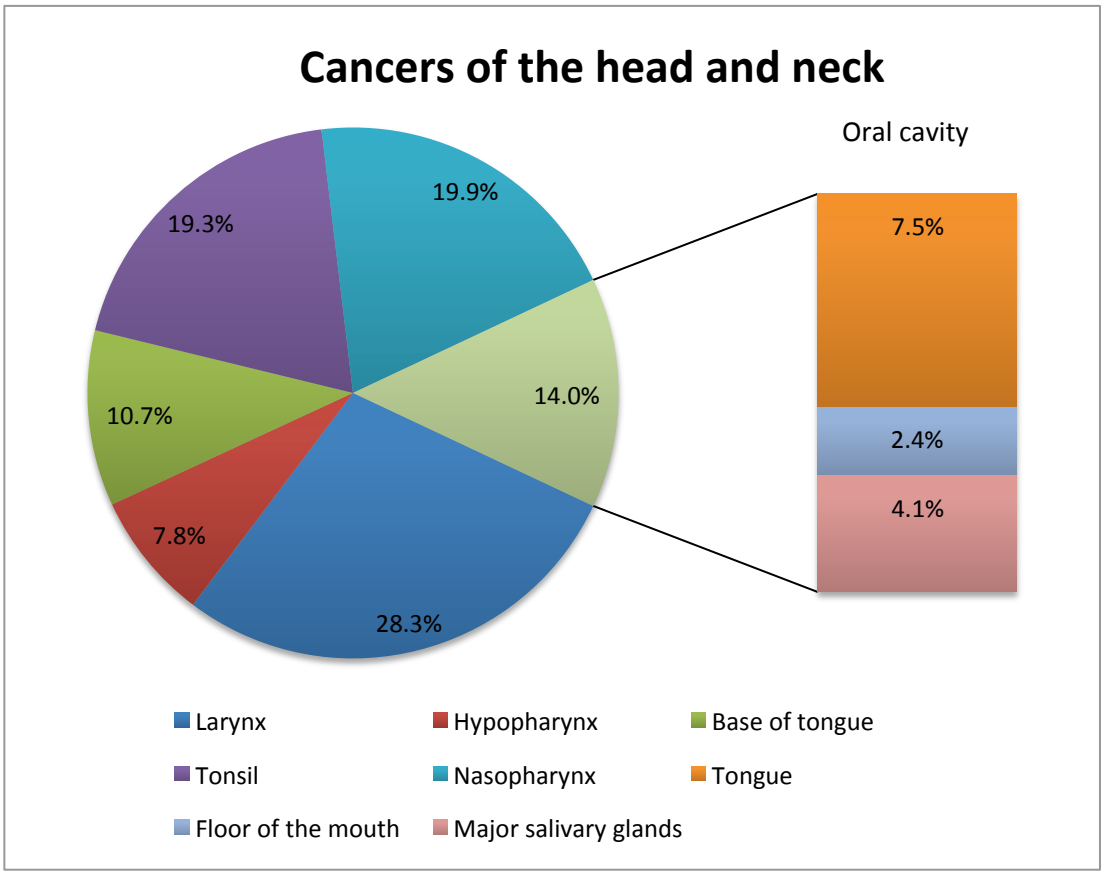

Figure 1-1 Estimated head and neck cancer incidence by site (Data from the National Head and Neck Cancer Audit 2014)

There remains a wide geographic variation in the epidemiology of HNC, incidence being more than twice in developing countries in comparison with more developed regions. This probably reflects differences in exposure to risk factors such as the use of smokeless tobacco products (betel quid) and areca nut (Joshi et al., 2014). The area characterised by high incidence rate is represented by Asia (57.6%), followed by Europe (20.3%) (Ferlay et al., 2012). In Europe age-standardised rates are higher in Central and Eastern Europe, followed by Western Europe and the Southern part of the continent (Stewart BW, 2014). High-risk regions for lip and oral cavity cancers include India and Pakistan, where the prevalence of areca nut chewing nationwide can be estimated to be around 30% (IARC, 2004) (Figure 1-2).

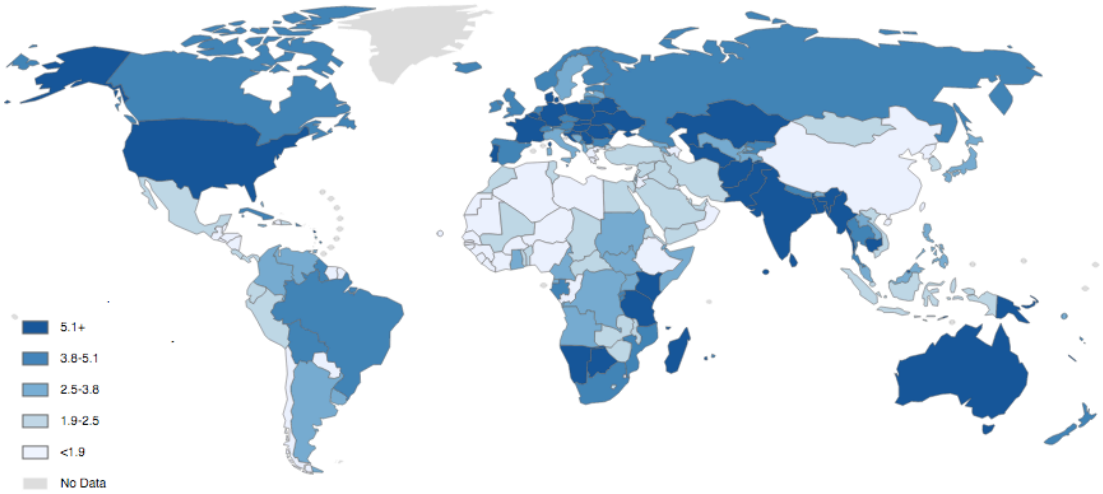

Figure 1-2 Global distribution of estimated age-standardized incidence rates (ASR) per 100 000, for lip and oral cavity cancer in both sexes (Reproduced from Globocan 2012)

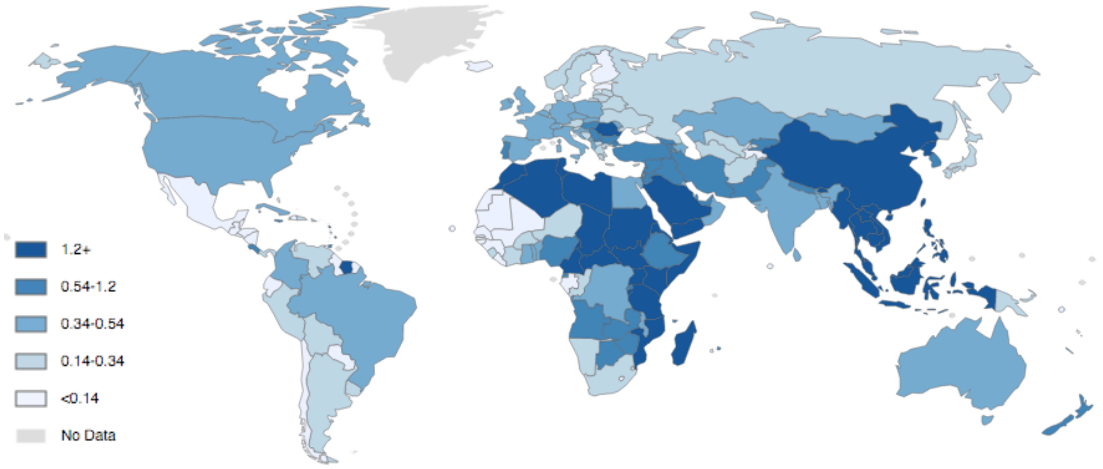

Figure 1-3 Global distribution of estimated age-standardized incidence rates (ASR) per 100 000, for nasopharyngeal cancer in both sexes (Reproduced from Globocan 2012)

Pharyngeal cancer is more prevalent in China and India than other countries such as United Kingdom and Italy (Ferlay et al., 2010) (Figure 1-3). The high prevalence of nasopharyngeal carcinoma in China may be due to a diet rich in salt-cured foods but there could also be a genetic predisposition, with first-degree relative of patients with nasopharyngeal carcinoma having an increased risk of developing the same disease by 7.6-fold (Cao et al., 2011).

For laryngeal cancer, central and eastern Europe, South America, and western Asia stand out (Figure 1-4).

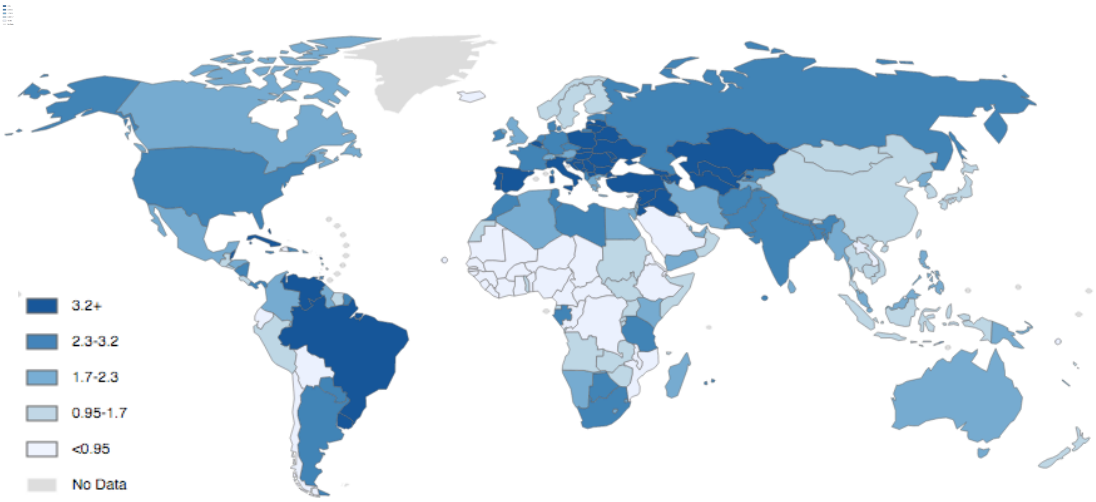

Figure 1-4 Global distribution of estimated age-standardized incidence rates (ASR) per 100 000, for laryngeal cancer in both sexes (Reproduced from Globocan 2012)

Overall, head and neck cancer remains a disease that typically affects the elderly, with 98% of patients aged over 40 and 50% aged over 60 in Europe (Mehanna et al., 2010). An increased incidence of oral and oropharyngeal squamous cell carcinoma among young individuals (18 to 44 years), however, has been reported in the last decade (Deschler et al., 2014). This trend change is likely linked to the increasing incidence of human papillomavirus (HPV)-related cancers.

The risk of developing HNC varies considerably with gender, with a male to female ratio of approximately 3:1 (Ferlay et al., 2012).

1.1.2 Risk Factors

The INHANCE consortium, established in 2004 to address epidemiological research questions, has confirmed that both tobacco smoking and alcohol

intake are established causes of oral, pharyngeal, and laryngeal cancer (Winn et al., 2015). Tobacco consumption is correlated with exposure to chemical carcinogens. The International Agency for Research on Cancer estimates that is sufficient evidence for carcinogenicity in either laboratory animals or humans for over 60 carcinogens contained in cigarettes (Alavanja et al., 2004). With regards to alcohol, research has focused on acetaldehyde, an intermediate metabolite of ethanol and a known carcinogen, which could stimulate carcinogenesis though inhibition of DNA methylation and interaction with retinoid metabolism (Seitz and Stickel, 2007). Although it has been proved difficult to obtain precise estimates of the risk because of the high correlation between smoking and drinking, it has been suggested that cigarette smoking in never drinkers of alcohol is strongly associated with an increased risk of head and neck cancer (OR=2.13, 95% 1.53-2.98), whereas the risk is doubled (OR=2.04, 95% CI 1.29-3.21) in people who never used tobacco but are alcohol heavy users (three or more drinks per day) (Winn et al., 2015). There is evidence that quitting smoking decreases the risk of head and neck cancer as soon as 1-4 years after stopping, whereas it takes 20 years to see the benefit of quitting alcohol drinking (Marron et al., 2010).

The link between viral infection and HNC has been firstly recognised in 1966, when the sera of patients with Burkitt's lymphoma and carcinoma of postnasal space were found to manifest precipitating antibodies against cells infected with the Epstein-Barr virus (EBV) (Old et al., 1966). EBV has been strongly implicated in the development of undifferentiated nasopharyngeal carcinoma, whereas its implication in keratinizing and non-keratinizing squamous cell carcinoma is controversial (Thompson and Kurzrock, 2004). Furthermore, it is now clear that HPV can induce carcinogenesis in the

oropharynx. The prevalence of HPV-related HNC is between 20-26%, with an higher prevalence of HPV-related tumours in the oropharynx (41%) compared to oral cavity (26%) and pharyngeal cancer (15%) (Abogunrin et al., 2014). HPV types 16, 18, 33 and 52 are associated with HPV-HNSCC, with HPV16 accounting for nearly 90% HPV-positive carcinomas of the oropharynx (Michaud et al., 2014, Syrjänen, 2010). The HPV-positive HNC patient seems to differ from the traditional patients, being younger and having no or relatively minimal exposures to traditional risk factors such as tobacco and alcohol. The risk of developing oropharyngeal carcinoma is, however, associated with a history of six or more lifetime sexual partners (OR 1.25), four or more lifetime oral sex partners (OR 3.36), and, for men, an earlier age at first sexual intercourse (OR 2.36) (Heck et al., 2010).

The strong association between HPV and head and neck cancer has received extraordinary attention because of the superior prognosis of HPV-driven cancers, which will be discussed in section 1.1.4.

Low education and income are also implicated in HNC development, with an almost 2-fold increased risk compared to intermediate and high education level (OR 1.61, 95%CI 1.13-2.31), the risk remaining elevated even after adjusting for recognized lifestyle behavioural risk factors (Conway et al., 2015).

Many studies have observed a protective effect of fruits (OR=0.52, 95% CI 0.43-0.62) and vegetables (OR=0.66, 95% CI 0.49-0.90) against developing head and neck cancer, which could be due to the presence of carotenoids and flavonoids with their anticarcinogenetic properties. The opposite seems true for higher intake of processed meat and notably for red meat (OR=1.40,

95%CI 1.13-1.74) (Chuang et al., 2012), due to iron over-storage and oxidative stress resulting from free radicals (Wiseman, 2008).

Family history of HNC is associated with an increased risk of developing cancer and a clear link can be seen in patients with Fanconi anaemia, an autosomal recessive genomic-instability syndrome associated with bone marrow failure, leukaemia, and congenital defects. In non-syndromic families, initial case–control studies have demonstrated a genetic predisposition, with first-degree relatives having a 1.7 fold increased risk of developing HNSCC (Negri et al., 2009).

1.1.3 Pathogenesis and Clinical Features

The initiation of head and neck cancer is a complex multistep process that entails a progressive acquisition of genetic and epigenetic alterations, leading to the inactivation of tumour-suppressor genes and/or activation of proto-oncogenes. Two independent research groups sequenced the whole-exome of approximately 100 HNC specimens (Stransky et al., 2011, Agrawal et al., 2011) and reported that tumour-suppressor activity is significantly more common than the activation of oncogenes.

Mutations were confirmed in genes that had been previously known to play a role in HNSCC, such as TP53, CDKN2A, EGFR, PIK3CA, PTEN, HRAS. Furthermore a novel mutation in NOTCH1 gene was reported in 10% to 15% of the HNSCC tumours.

TP53 gene, a tumour suppressor gene playing a key role in the response to DNA damage and oncogenic stress, is mutated in 47-60% (Agrawal et al., 2011, Pickering et al., 2013) cases of HNC. Mutations in TP53 are acquired early in the pathogenesis of HNC and can be observed also in pre-malignant

lesions, but it is still unclear whether alterations in TP53 are prognostic for overall survival (Riaz et al., 2014).

Inactivation of p53 can also occur as a result of inactivation of the tumour-suppressive CDKN2A (Cyclin-Dependent Kinase Inhibitor 2A) gene, seen in 30% of HNSCC, with the gene being mutated in another 10% of samples (Riaz et al., 2014). Among other proteins, CDKN2A gene provides instruction for p16 and p14 proteins, tumour-suppressor proteins involved in the regulation of cell growth and division.

The next most commonly altered gene in HNSCC is the gene encoding for protein cyclin D1 (CCND1), essential for the facilitation of cell cycle progression. The CCND1 gene is amplified in over 20% of tumours (Cerami et al., 2012).

The epidermal growth factor receptor (EGFR) is a member of the HER/erbB family of receptors and its activation through binding of its ligand leads to a downstream signalling cascade. EGFR is expressed in 90% or more of cases of HNSCC, with gene amplification observed in 10-30% of them (Suh et al., 2014).

The phosphoinositide 3-kinases (PI3Ks) family plays a pivotal role in cellular regulatory mechanisms. Genetic mutations of the PI3K pathway, notably in the PIK3CA gene, have been reported in 6% to 11% head and neck tumours (Agrawal et al., 2011, Pickering et al., 2013). Additionally in 3% of HNSCC has been observed a loss of the tumour suppressor PTEN (phosphatase and tensin homolog), which regulates the cellular level of activated kinases, key messengers in the regulation of cellular processes. Loss of PTEN results in unrestrained signalling by the PI3K pathway.

Ras proteins are ubiquitously expressed in all cell lineages and are involved in transmitting signals within cells (cellular signal transduction). Mutations in the Ras proto-oncogenes, a binding protein localised on the plasma membrane have been found in 4–5% of HNSCC cases (Suh et al., 2014).

A promising new therapeutic approach is represented by the recent discovery of mutations in NOTCH1 gene, encoding for a transmembrane receptor that plays a role in cell differentiation and embryonic development, seen in 18% of HNSCC cases (Sun et al., 2014).

Very recently The Cancer Genome Atlas Research Network has uncovered a different mutational profile of HPV-positive cancers which are characterised by a better prognosis with respect to their HPV-negative counterparts (Lawrence et al., 2015). Many patients with HPV-positive cancers have specific alterations of the gene FGFR3 and mutations in the PIK3CA gene, whereas the EGFR and p53 are rarely altered in HPV-positive malignancies. This study has potentially important clinical implications, offering novel diagnostic and treatment directions.

Histopathologically head and neck cancer development comprises progressive stage of hyperplasia, dysplasia, carcinoma in situ and invasive carcinoma, with progressive loss of maturation, architectural disorganization, increasing pleomorphism and cell size (Argiris et al., 2008).

Clinical features and symptoms of HNC can vary greatly depending on affected anatomical site. Early oral cancer might present as a white hyperkeratotic plaque (leukoplakia), a mucosal red lesion (erythroplakia), mixed red and white lesion (erythroleukoplakia), as well as an indurated lump or ulceration with fissuring or raised exophytic margins. Advanced oral

squamous carcinomas are associated with a wide range of extra-oral and intra-oral signs and symptoms, including large ulcerated mass, bleeding, pain, earache, dysphagia, ankyloglossia, trismus and paraesthesia (Scully and Bagan, 2009).

In Europe and USA, the tongue is the most common intra-oral site, accounting for 40% of oral cancers, whereas cancers arising in the buccal mucosa are common among Asian populations due to betel quid/tobacco chewing habits. Other sites for oral cavity cancer include floor of mouth, gingivae and palate. The lip is involved more frequently in Canada and Australia, with a strong link to chronic exposure to sunlight (Warnakulasuriya, 2009).

Oral cancer can arise from clinically evident mucosal lesions (potentially malignant disorders) via progressive degrees of pre-malignant epithelial changes (Warnakulasuriya et al., 2007). The annual rate transformation of premalignant lesions ranges from approximately 1-4% for oral leukoplakia (Warnakulasuriya and Ariyawardana, 2015), to a higher risk for erythroplakia, for which is not possible to calculate a reliable annual malignant transformation due to the rarity of the disorder (estimated prevalence 0.02-0.83%) (van der Waal, 2009). Oral lichen planus, a chronic inflammatory disease of unknown aetiology, is an oral disease associated with a 2% increased risk of developing malignancy (Landini et al., 2014).

Early diagnosis in oropharyngeal and pharyngeal cancer is rare, with most patients presenting with loco-regionally advanced disease and cervical metastases (Wee, 2015).

Patients with oropharyngeal cancer may experience a foreign body sensation, soreness, and discomfort in the throat or altered voice. More advanced lesions can lead to earache, throat pain, ankyloglossia, dysarthria and dysphagia (Cohan et al., 2009).

Laryngeal cancer can manifest with hoarseness, sore throat, dysphagia, odynophagia, earache, haemoptysis and dyspnoea due to the vocal cord fixation (Marioni et al., 2006).

1.1.4 Staging and Prognosis

Therapy for head and neck cancers has evolved over the past decade and a significant improvement in 5-year disease-specific survival from 55% in 1992-1996 to 66% in 2002-2006 has been observed, although the change in survival does not seem to include laryngeal cancer (Howlader N, 2015). The most important prognostic factors of HNC include staging, site, local invasion, angioinvasion, and HPV status.

The Classification of Malignant Tumours (TNM) is a cancer staging notation system used to indicate the tumour stage (T), nodal status of the neck (N), and the presence of metastases (M), representing strong prognostic factors in head and neck tumours. The Table 1-1, adapted from the English cancer networks DAHNO (Data for Head and Neck Oncology) clearly shows how survival rates are significantly better for patients diagnosed with early stage cancer at both one year and three years after the diagnosis (Watters, 2011).

Table 1-1 Comparison of survival rates for head and neck cancers diagnosed in 2004-2006 (adapted from DAHNO report 2011)

Site	Cases	One year relative survival rates in %	Three years relative survival rates %
Oral Cavity	1592	79.9	61.4
Early	574	93.8	80.5
Late	616	68.9	45.4
Not Known	402	76.2	58.2
Oropharynx	519	80.9	62.5
Early	68	85.4	71.8
Late	307	82.1	68.0
Not Known	144	76.2	59.0
Hypopharynx	150	62.7	32.7
Nasopharynx	47	93.4	60.6
Larynx	1552	88.9	74.1
Early	587	97	89
Late	439	75.9	50.5
Not Known	526	88.9	74.1

Tumour site is a strong prognosticator of survival, regardless of treatment modality (Ries LAG, 2007).

The prognosis of patients with HPV-positive HNSCC is widely discussed in the literature. The RTOG 0129 trial has offered a significant insight in the prognostic value of HPV infection, retrospectively analysing a randomised clinical trial (RCT) where patients with stage III-IV oropharyngeal carcinoma were randomised to accelerated-fractionation or standard fractionation radiotherapy. The study revealed that patients with HPV-positive tumour had a 25% improvement in 3-years survival rate compared with HPV-negative tumour (82.4% vs. 57.1%) (Ang et al., 2010). Two years later a meta-analysis confirmed a 54% improvement in overall survival rate for HPV-positive HNSCC compared with HPV-negative HNSCC (O'Rorke et al., 2012). The characteristics of population affected from HPV-HNSCC, with fewer comorbidities, younger age and limited exposure to risk factors cannot entirely explain their prognostic advantage, since after adjustment of these confounders there was a persistent difference in survival among patients with

HPV-positive and HPV-negative tumours. Indeed HPV-positive tumours tend to respond better than HPV-negative tumours to platinum-based induction chemotherapy regimens and radiation therapy independently from the radiation regimen, the addition of radiation sensitizers, or the addition of chemotherapy (Benson et al., 2014).

Patients' general health status, including comorbidities and age, can also influence the prognosis, as these factors affect treatment delivery. The impact of comorbidity on prognosis has been confirmed in a systematic review including 31 studies. A quantitative analysis of 10 of these studies showed a significant difference in mortality in patients with comorbidities (mainly liver diseases and diabetes in the young group and cerebrovascular and cardiovascular in the older group) compared to otherwise healthy patients (HR 1.38; 95% CI: 1.32–1.43) (Boje, 2014). However it could be argued that clinical trials often tend to exclude patients with comorbidities to avoid the confounding influence on the outcome whereas it would be sensible indeed to include these patients to truly evaluate their prognosis.

1.1.5 Quality of Life in head and neck cancer survivors

The World Health Organization defines the Quality of Life (QoL) as "an individual's perception of their position in life, in the context of the culture and value system in their life, and in relation to their goals, expectations, standards and concerns" (Kuyken, 1995).

A diagnosis of head and neck cancer can have a negative impact on quality of life caused by the fear of altered appearance, changes in the ability to speak, swallow and breath, and high levels of symptomatology (pain, xerostomia, shoulder dysfunction) (Semple et al., 2013). The reported

prevalence of psychological distress amongst head and neck cancer patients varies from 12% to 45%, depending on the criteria and diagnostic tool used for the assessment (Haman, 2008). Patients with HNC experience deterioration in global QoL scores immediately after treatment, with a gradual return to a baseline level for the majority of distresses within one year after treatment completion. However, at 12 months xerostomia and fatigue are not returned to normality, and can per se affect patients' QoL, as it will be explained in paragraph 1.3.8 (Babin et al., 2008).

The site of tumour is also an important determinant for QoL. Oropharyngeal cancer is associated with lower QoL score, likely due to advanced stage at presentation (Rogers et al., 2007).

There remain conflicting data regarding the influence of gender upon QoL, with more pronounced fatigue, general pain and salivary impairment being reported in women than in man (Bjordal et al., 2001).

A different trend in QoL has been reported in patients of different age groups, with poorer QoL scores and more symptoms as fatigue, social eating in older patients when compared to younger patients, who mainly complained of insomnia, nausea and financial difficulties (So et al., 2012).

Treatment modality has a profound impact on QoL outcome: combined chemoradiotherapy leads to a worse QoL compared with radiotherapy (RT) alone, whereas intensity modulated radiotherapy (IMRT) yields better QoL compared with conventional or three-dimensional conformal radiotherapy (Klein et al., 2014).

1.1.6 The curative treatment for head and neck cancer

Head and neck cancer treatment is determined in a multidisciplinary setting. Early-stage diseases can be treated with either surgery or radiotherapy depending on the primary tumour site, with cure rates of 70–90%. Radiotherapy is the treatment of choice when function preservation is of paramount importance, such as carcinoma of the larynx and tongue base (Argiris et al., 2008).

Unfortunately the majority of patients present with locally advanced stage disease which requires a multimodality therapy (Suh et al., 2014).

1.1.6.1 Surgery

The aim of HNC surgery is the complete surgical resection with microscopic clearance of the tumour. The surgical procedure has the advantage of providing tumour histology and achieving effective local tumour control. Over the past three decades there has been a change in the treatment of HNC, with the use of modern surgical technique and a trend toward an increased use of non-surgical treatment modalities in order to achieve organ preservation and optimise patients' quality of life (Hartl et al., 2013).

For early stage (I-II) HNC local excision of the tumour and a clinical margin of one centimetre of healthy surrounding tissue is usually recommended. Early HNC have an excellent prognosis, with cure rate of 80% for stage I disease and 65% for stage II (Kalavrezos and Bhandari, 2010).

For advanced HNC, a combination of surgery and radiotherapy or the use of chemoradiotherapy (CRT) offers the highest chance of achieving cure (Roland, 2011).

The presence of cervical lymph node metastases is a key prognostic factor in patients with HNC and neck dissection is usually performed with a tendency to preserve non-lymphatic structures. The recognition that shoulder and neck dysfunction after neck surgery is an important contributor to quality of life after treatment has led to a progression from radical to selective neck dissection, where one or more of the lymphatic groups normally removed in the radical neck dissection is preserved (Medina and Krempl, 2012).

Several techniques are being tested to improve functional outcomes, such as the Transoral Robotic Surgery. While there is no doubt on the advantages of transoral techniques over open techniques, it is still controversial whether the oncologic outcomes are equivalent or superior to results of other surgical and nonsurgical treatments (Moore et al., 2012).

1.1.6.2 Chemotherapy

As recently as the late 1980s, the role of chemotherapy in head and neck cancer was predominantly limited to palliation of recurrent or distant metastatic disease. Since then, chemotherapy has demonstrated to play a key role in the management of local or regionally advanced head and neck cancers. Furthermore, following a growing understanding of head and neck cancer molecular biology, new anticancer agents have been approved by the U.S. Food and Drug Administration (FDA). Cetuximab, a monoclonal antibody targeting the EGFR, has been approved for use in locoregionally advanced and recurrent disease. Docetaxel, an anti-mitotic chemotherapy agent, can be given along with cisplatin and 5-fluorouracil, agents which have been available for the treatment of cancer for more than 2 decades.

For many years, chemotherapy has been administered in the neoadjuvant setting (before radiotherapy) or adjuvant setting (after radiotherapy) and, more recently, concurrently with radiotherapy.

One of the first studies on chemotherapy, the Veterans Administration Larynx Preservation Study, showed in a prospective randomized study that over 300 patients with laryngeal squamous cell carcinoma (Stage III-IV) treated with induction chemotherapy followed by definitive radiation therapy had similar survival outcome to those who had conventional laryngectomy and postoperative radiation. It is important to highlight, however, that 36% of the patients in the chemotherapy group required total laryngectomy to a later stage (Wolf et al., 1991).

Since this pioneering work, several studies have confirmed the additional benefit of chemoradiotherapy with regards to HNC local control and survival rates with the greatest survival benefit seen with the concurrent administration of chemotherapy and radiotherapy. The results of 87 randomised trial have been combined in a meta-analysis (Blanchard et al., 2011), which showed a significant overall survival benefit for concomitant chemotherapy at five years of 8.9%, 8.1%, 5.4%, and 4.0% for oral cavity, oropharynx, larynx and hypopharynx tumours respectively in comparison to loco-regional treatment alone. Of note, the benefit was largely confined to concomitant chemotherapy with no clear evidence of a benefit for induction and adjuvant chemotherapy. The original meta-analysis excluded nasopharyngeal cancers, but a significant overall benefit in survival of 6.3% at 5 years when compared to radiotherapy alone has been showed in their recent updated version (Blanchard et al., 2015).

Promising new molecular targeted therapies in head and neck oncology include EGFR monoclonal antibodies.

Cetuximab, the most studied antibody has shown encouraging results in a phase II study on advanced head and neck cancer (3-year overall survival of 76%), although an unexpected rate of death and adverse events led to early closure of the study (Pfister et al., 2006). In a landmark study by Bonner et al., cetuximab improved local control and survival (49.0 vs. 29.3 months) in 424 patients with locoregionally advanced head and neck cancer randomly assigned to treatment with cetuximab and radiotherapy or radiotherapy alone (Bonner et al., 2006). The results of this study should be interpreted with caution, as the control group did not receive the standard of care (chemoradiotherapy) but only radiotherapy. Several clinical trials have addressed this issue comparing cetuximab to standard chemoradiotherapy regimen. The phase III study RTOG 0522 randomized patients to platinum based chemoradiation with or without cetuximab (Ang et al., 2014), failing to show any difference in progression-free survival between arms. Additional studies comparing chemoradiation to cetuximab with radiation are on-going (Cuneo et al., 2015).

In addition to cetuximab, researchers have also focused their attention on the fully humanized monoclonal anti-EGFR antibody panitumumab. In the phase II study CONCERT-1 patients with locally advanced HNSCC were randomized to chemoradiation therapy with or without panitumumab (Mesía et al., 2015). The study showed a worse locoregional control rate (61% vs. 68%) and a higher toxicity when panitumumab was added to the treatment (43% vs. 32%). In 2015 Giralt et al published the results of CONCERT-2, an

open-label, randomised trial comparing the treatment with panitumumab plus radiotherapy with platinum-based chemoradiotherapy in 152 participants over 22 sites. The results of the study clearly showed the superiority of cisplatin therapy over panitumumab for unresected stage III-IVb squamous cell carcinoma of the head and neck in terms of loco-regional control at 2 years (61% vs. 51%) and adverse events (Giralt et al., 2015).

1.1.6.3 Radiotherapy

Radiotherapy is a highly effective treatment modality for HNC, either as single or combined treatment.

Radiotherapy has been dramatically revolutionised by the introduction of IMRT, where radiotherapy beam's intensity can be optimised to deliver a high dose of radiation to specified volumes, reducing the incidence and severity of radiation-associated side effects.

Particle therapy such as protons therapy may allow additional advantages to patients with tumours close to particularly radiosensitive organs (brain, spinal cord), but these treatment modalities need to be validated in prospective clinical trials.

1.2 Radiotherapy for Head and Neck Cancer

1.2.1 The 4 R's of radiobiology

The relationship between the dose delivered to the tumour and the probability of tumour control is sigmoidal: below a threshold dose there is no evidence of tumour control whereas beyond this limit the higher the radiation dose delivered to the tumour the higher the tumour control (El Naqa et al., 2006). Increasing the dose, however, would also increase the probability of normal tissue complication.

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Radiotherapy has been used in the treatment of cancer for its ability to damage the DNA of cells leading to cell death. The damage to DNA can be direct, when the incident photon displaces an electron from the target molecule, or it can be the result of indirect ionization, if another molecule, such as water, is ionized and damages the DNA.

Indirect ionization produces unstable components known as free radicals, able to interact with DNA resulting in either single-stranded or, most frequently, in double-stranded DNA breaks. Free radicals are in part inactivated through scavenging reactions (Caudell, 2012).

Radiotherapy dose is prescribed in units of Gray (Gy), a measure of the amount of energy deposited in the tissue. For one Gy of absorbed radiation dose, there are in excess of 10^5 ionizations within the volume of every cell and more than 1000 damages to DNA bases (Bradly et al., 2009).

The radiation dosage can be delivered in fractionated small daily portion to take advantage of the cell kill process of radiation treatment, originally described by Withers with the 4 R's of radiobiology: repair of sublethal DNA damage, cell repopulation, redistribution of cells in the cell cycle, and reoxygenation of previously hypoxic tumour areas (Withers, 1999). Later, the principle of radiosensitivity was introduced (Steel et al., 1989).

By fractionating radiation treatment over 5 to 7 weeks, normal cells are able to *repair sublethal damage* and repopulate, limiting the damage to the normal tissue. Conversely, malignant cells have often suppressed repair pathways, often through mutation or inhibition of TP53, preventing them from

undergoing efficient repair. *Repopulation* of tumour cells may be an important cause of treatment failure. Fractionation regimen allows eradication of the original tumour cells and also of any formed in the interval between fractions. Additionally fractionation overcomes the different tumour sensitivity to radiation, which varies during the cell cycle (*redistribution* of cell along the cell cycle), and kills the radiosensitive well-oxygenated cells, exposing the relative hypoxic-radioresistant cells (*reoxygenation*) (Marcu, 2010).

1.2.2 Radiotherapy regimens for head and neck cancer

Traditional RT regimen in head and neck oncology consists of a daily fraction dose of 1.8-2 Gy, five fractions per week, for seven weeks (conventional fractionation). Total dose can vary from 60 to 70 Gy (Fletcher, 1988).

Over the past few decades new radiotherapy regimens have been designed and tested in order to improve tumour control and limit toxicity. The most commonly used altered fractionation schedules are hyperfractionated, accelerated and hybrid accelerated-hyperfractionated radiotherapy.

Hyperfractionation involves multiple daily fractions (2 or 3) of small doses (under 1.8 Gy) with an interval between daily fractions of at least 6 hours to allow normal tissue repair. The aim of the hyperfractionation approach is to deliver a higher total dose of radiation, but in smaller doses, thus minimising the late side effects of the treatment. With accelerated fractionation, the total number of fractions is delivered over a shorter number of elapsed days, in order to counteract the rapid repopulation of tumour cells during the course of radiotherapy and improve locoregional control (Bernier and Bernier, 2011).

Several trials have studied the role of unconventional fractionated radiotherapy in head and neck squamous cell carcinoma. The European

Organization for Research and Treatment of Cancer (EORTC) 22791 trial compared a radiotherapy regimen of 1.8- to 2.0-Gy fractions (total dose 70 Gy) delivered once daily to a total dose of 80 Gy with 1.15-Gy fractions delivered twice daily for the treatment of T2–T3 oropharyngeal carcinomas, excluding base of the tongue. The authors observed a higher number of disease-free people at 5 years in the hyperfractionated arm (59%) compared the conventional arm (40%) (Horiot et al., 1992), although the benefit was not observed in T2 patients. The RTOG 9003 trial tested conventional fractionation, hyperfractionation (total dose 81.6 Gy), and two regimens of accelerated fractionation (67.2 Gy in 1.6 Gy fractions twice a day or 72 Gy in 1.7 Gy fractions). The recently published final report showed an improved overall survival (HR 0.81) only for the hyperfractionation regimen, without increase of late toxicity (Beitler et al., 2014). Positive results for the hyperfractionation regimen were also reported in Brazil in a clinical trial randomly assigning 112 patients with stage III or IV oropharyngeal cancer to hyperfractionation (70.4 Gy in 1.1 Gy twice a day) or conventional radiotherapy (66Gy in 2.0 Gy daily). The overall survival rate at 42 months was 27% for the hyperfractionation arm and 8% for the conventional radiotherapy (Pinto et al., 1991).

Accelerated fractionation in head and neck malignancies also has been tested in several clinical trials. The largest randomised study of accelerated fractionation is the Danish Head and Neck Cancer Study Group (DAHANCA) where almost 1500 patients received conventional fractionation or accelerated fractionation (six fractions per week) with the same number of fraction and dose per day (1 fraction of 2 Gy) and total dosage (66 Gy) between groups. Overall 5-year local-regional control (LRC) rates were 70%

and 60% for the accelerated and conventional groups respectively, with no difference in survival but significantly worse acute morbidity in the experimental group (Overgaard et al., 2003). The same study design was applied by the same research team few years later in developing countries (the IAEA-ACC study) and similar results were obtained (5- year LRC of 42% versus 30% in the conventional group) (Overgaard et al., 2010).

Hybrid accelerated-hyperfractionated radiotherapy was developed to combine the two altered radiotherapy regimens. Although local control was improved in the GORTEC regimen, accelerated radiotherapy with a further dose reduction did not show a similar benefit in the CHART trials. The phase III clinical trial CHART (Continuous Hyperfractionated Accelerated Radiotherapy) observed similar tumour control rate in 918 patients randomized to 54Gy in 36 fractions of 1.5Gy (over 12 days) or to conventional radiotherapy (Dische et al., 1997). An improvement in local tumour control rate but at a prize of an increased incidence of acute mucositis was reported in the GORTEC study, where 268 patients were randomised to hybrid accelerated fractionation radiotherapy (62-64 Gy in 31-32 fraction over 3 weeks) or conventional radiotherapy. This study reported a difference of 24% in the LRC in the experimental arm compared to the control arm. Severe toxicity, however, was observed in the accelerated group, with most of the patients having difficulties in swallowing liquid, and requiring a feeding tube (Bourhis et al., 2004).

A meta-analysis of 6,515 patients across 15 trials assessed survival and local control rate between hyperfractionated, accelerated, hybrid accelerated with total dose reduction and conventional radiotherapy (Baujat et al., 2010). The

meta-analysis showed a significant survival benefit with altered fractionated radiotherapy, corresponding to an absolute benefit of 8% at 5 years for hyperfractionated radiotherapy, 2% with accelerated radiotherapy without total dose reduction and 1.7% with total dose reduction.

A second meta-analysis described the potential benefit of altered fractionation in the treatment of unresected locally advanced HNSCC (Budach et al., 2006). This study confirmed a significant improvement in overall survival with hyperfractionated radiotherapy (14.2 months) compared to conventionally fractionated radiotherapy, but not with accelerated regimen.

Conformal 3D radiotherapy and IMRT

The close proximity of HNC to tissue at risk of severe damage (spinal cord, brainstem, parotid glands, lacrimal glands, eyes and optic tract) led to the development of new RT technologies delivering a radiation dose that conforms to the target volume and decreases exposure of other structures, the three-dimensional conformal radiotherapy (3D-CRT). The ability of 3D-CRT in sparing salivary glands tissue and therefore limiting the incidence and severity of radiation-associated xerostomia has been confirmed in clinical trials (Portaluri et al., 2006, Wiggenraad et al., 2005).

An advanced approach to three-dimensional treatment planning is the IMRT, which uses non-uniform radiation beam intensities to increase the delivery of radiation to the tumour while minimizing the dose to adjacent normal tissues.

IMRT

IMRT allows simultaneous delivery of individualized dose levels to the targets and elective nodal areas by modulating the intensity of the radiation beam.

IMRT has been used since 1996 to limit the radiation dose to critical organs in head and neck cancer patients. With IMRT the radiation dose to salivary glands is reduced, with the contralateral parotid gland receiving on average 20 Gy (Bhide et al., 2012). This dosage well correlates with the observation that minimal gland function reduction occurs when the mean dose is less than 15 Gy, with gland function gradually reducing at radiation doses of 20–40 Gy, and strongly reducing (usually by >75%) at >40 Gy (Deasy et al., 2010).

A systematic review of five randomised trials (871 patients) showed that IMRT can significantly provide better tumour target coverage than two-dimensional conventional radiotherapy (2D-CRT) and 3D-CRT (HR of 0.76; CI 0.66-0.87) (Marta et al., 2014).

More than 20 clinical studies have been published in the literature reporting the clinical effect of IMRT on xerostomia or salivary function, but less than a quarter of them were randomised clinical trials. The pioneer was Pow in 2006 who compared the treatment with IMRT and conventional radiotherapy in 51 patients with nasopharyngeal carcinoma (Pow et al., 2006). IMRT proved to be better than RT in terms of parotid gland sparing, with 50% of IMRT patients recovering at least 25% of baseline salivary flow compared to 5% in the control arm. No specific outcome measure for the assessment of xerostomia was used in this study. He was followed by Kam et al in 2007 who randomly assigned 56 patients with nasopharyngeal carcinoma to either IMRT or 2D-RT. He observed a lower incidence of severe clinician-rated xerostomia (RTOG) in the IMRT arm 1 year after treatment completion (39.3% vs. 82.1%) (Kam et al., 2007). No significant difference in the

reduction of patient-rated xerostomia has been observed between the two arms at any time point.

Comparative studies have also confirmed that IMRT can significantly reduce the severity and prevalence of xerostomia in head and neck cancer survivors. Lee et al. (Lee et al., 2006) in a prospective non -randomised study and Lambrecht et al (Lambrecht et al., 2013) in a retrospective study, observed a similar reduced incidence of xerostomia ≥ grade 2 for IMRT vs. accelerated concomitant boost radiotherapy at 20 months and vs. 3D-CRT at 6 months (12% versus 67% and 23% versus 68% respectively). Undoubtedly the landmark clinical trial providing the strongest evidence regarding the benefit of IMRT in reducing salivary gland damage has been performed by Nutting and published in Lancet in 2011 (Nutting et al., 2011). In this study 94 patients with pharyngeal carcinoma were randomly assigned either to IMRT or conventional RT among six UK radiotherapy centres. Clinician-rated xerostomia (LENT-SOMA) 12 months after treatment completion was observed in 38% of patients treated with IMRT versus 74% in the control group. Significant differences were also noted in unstimulated and stimulated parotid gland sialometry. No statistically significant difference between groups has been noted with regards to subjective xerostomia symptoms and quality of life.

Although the consistent benefit offered by IMRT in achieving salivary glands sparing, this did not necessarily translate into significantly better QOL outcomes. The largest cross-sectional study evaluating post-radiotherapy QoL in 640 head–neck cancer survivors undergoing conventional, 3D-RT and IMRT, showed a benefit in global QoL and physical functioning when

comparing IMRT and conventional radiotherapy, however the benefit was not confirmed when IMRT was compared to conformal 3D-CRT (Wan Leung et al., 2011).

Despite several studies focusing their attention on parotid-sparing technique, which is now the standard radiotherapy technique for HNSCC patients, there are no randomized trials evaluating the clinical outcomes of sparing the submandibular glands (SMGs), which contribute for 65-90% to the unstimulated salivary flow (Gensheimer et al., 2014).

Very limited evidence from two small reports suggested that SMG-sparing IMRT could reduce long-term xerostomia compared with non-SMG sparing IMRT (Saarilahti et al., 2006, Wang et al., 2011). Saarilahti et al treated 36 patients with either parotid-sparing IMRT or parotid-sparing and SMG-sparing IMRT observing that at 12 months there were fewer patients reporting grade 2 or worse xerostomia (4 vs. 11) and they had better salivary gland function (60% vs. 25% of the baseline value) in the combined RT group. Their results were partly confirmed by Wang et al in a longitudinal study on 52 patients treated with or without contralateral submandibular gland sparing. Mean salivary flow at 2, 6, 12, and 18 months after RT and clinician rated xerostomia (RTOG) at 2 and 6 months were better in the group who had the submandibular salivary gland spared. However the results were not stratified for grades of xerostomia severity, which makes unclear the real benefit of this approach.

Future approach

There has been considerable interest in the use of radiotherapy based on protons, charges particles with unique physical properties, in order to further

reduce the toxicity of head and neck radiotherapy (Lukens et al., 2015). Initial dosimetric studies have suggested that proton radiotherapy could allow dose escalation without exceeding the tolerance of critical structures (Cozzi et al., 2001) and the lower radiation doses delivered to the parotid glands could lead to complete recovery for both parotid glands (Widesott et al., 2008).

Although randomised clinical trials are needed to understand whether the dosimetric advantage of proton therapy can translate into clinical benefit, preclinical models suggested that the reduced dose to the muscles involved in swallowing mechanisms and to the posterior fossa could lead to a reduction of the incidence of grade 2 or worse dysphagia (8%) (van der Laan et al., 2013) and fatigue (Gulliford et al., 2012). Of note, fatigue was the only adverse event more prevalent in the IMRT group compared to conventional radiotherapy (74% vs. 41%) in the cited study performed by Nutting (Nutting et al., 2011).

1.2.3 Oral adverse side effects of radiotherapy to the head and neck

Head and neck cancers can require high doses of radiotherapy on large areas including the oral cavity, maxilla, mandible and salivary glands and are therefore associated with several adverse reactions (Jham and Freire, 2006). Oral adverse side effects of radiotherapy to the head and neck will depend on the volume and area being irradiated, on the total dose of radiation.

Functionally, tissue organization has been divided into parallel or serial structure, which has an effect on the radiation effects (Hodapp, 2012). A parallel organ, such as the parotid gland, can lose its functionality only if all the subvolumes of the organs are damages, whereas a serial organ can be

damaged even if only one of its subvolumes is damaged. As a consequence it is important to preserve all the functional units of serial organs (e.g. spinal cord) in order to guarantee the functionality of the tissue. This concept of tissue organization is of paramount importance during treatment planning.

Normal tissues exhibit acute toxicity (during or right after treatment) and late toxicity (months or years) after cancer treatment. Acute toxicity typically reflect cell killing of rapid renewal systems such as skin, mucosa, or bone marrow and they are normally reversible. Conversely late toxicities are more complex, typically involving slow renewal systems, potentially causing significant tissue damage depending on the organ involved. Late complications are normally irreversible, leading to permanent incapability and to a worsening of quality of life.

Oral adverse side effects of radiotherapy to the head and neck include oral mucositis, osteoradionecrosis, trismus and xerostomia.

Oral mucositis

Oral mucositis is the most common acute side effect resulting from exposure to chemotherapeutic agents and ionizing radiation. Severe oral mucositis occurs in 29-66% of all patients receiving radiation therapy for HNC (Satheesh Kumar et al., 2009).

Oral mucositis is defined as an inflammation of oral mucosa manifesting with erythematous and ulcerative lesions (Raber-Durlacher et al., 2010). The lower lip, tongue, floor of the mouth, and soft palate are affected areas. The ulcers can be multiple and extended and, by offering a ready portal of entry for microorganisms, can increase the risk of systemic infection.

Radiation-associated mucositis typically begins from the second week of radiotherapy through cumulative doses of around 15 Gy, with ulcerative mucositis noted at doses of 30 Gy.

The severity and the clinical manifestations of oral mucositis can vary depending on the dose of radiation, the association of radiotherapy with chemotherapy and the chemotherapeutic agent used. The risk of developing severe oral mucositis for cancers to the oropharynx, hypopharynx and larynx is approximately 34%, 57% and 43% respectively (Trotti et al., 2003). It is unclear whether IMRT technique can achieve a reduction in the incidence of acute mucositis, with a study reporting a reduction of 32% (Kouloulias et al., 2013), and other studies showing comparable rates to patients receiving 2/3D-RT (Kruser et al., 2013).

Mucositis has been described as a complex biological process consisting of five sequential phases (Sonis, 2011).

In the first phase (initiation) the direct wound to the DNA of the basal layer cells in the epithelium takes place with the appearance of reactive oxygen species. In the second phase (signalization) reactive oxygen species-activated enzymes induce apoptosis, with an increased production of the proinflammatory cytokines. The third phase (amplication) is characterized by the loss of mucosa integrity, leading to the ulceration (fourth phase). Finally a signal from submucosal tissue allows renewed cellular proliferation and healing.

The Multinational Association of Supportive Care in Cancer reviewed the evidence available in literature and developed guidelines for the management of oral mucositis. Interventions with high level of scientific

evidence than can be recommended are benzydamine mouthwash, cryotherapy, recombinant human keratinocyte growth factor and low-level laser therapy (Lalla et al., 2014).

Osteoradionecrosis

Osteoradionecrosis (ORN) is a severe and debilitating complication following head and neck radiotherapy. ORN is defined as an area of exposed irradiated bone that fails to heal over a period of 3-6 months in the absence of local neoplastic disease (Marx, 1983).

The incidence of ORN following head and neck radiotherapy ranges from 4.74%- 37.5% (Nabil and Samman, 2012), although it is significantly declined after the introduction of dental assessment before undergoing radiotherapy and advances in RT technique. Grade 3 ORN (the grade defines the need of mandibular resection) has been shown to be substantially reduced (to <1%) by the use of intensity-modulated radiation therapy (IMRT) techniques (Studer et al., 2006); whereas grade 1–2 ORN was reported to be <10% in patients at risk (radiation therapy with doses of ~70–72 Gy for tumour close to the mandible) (Studer et al., 2015).

Mandibular ORN is more prevalent when compared to the maxilla due to the relatively poor vascularisation and to the dense structure of mandibular bone (Thorn et al., 2000). ORN can occur spontaneously or after trauma induced by dentures or dental surgery (Jiang et al., 2014).

Pathogenesis of ORN remains unclear; the most accredited theory suggests that the necrosis is triggered by vascular insufficiency, radiation-associated fibrosis and acute inflammation (Lyons and Ghazali, 2008). Three phases

have been described: a pre-fibrotic phase characterised by chronic inflammation inducing fibroblastic activation, a constitutive organised phase with disorganisation of the extracellular matrix and a late fibroatrophic phase, featuring fragile and poorly vascularized tissue (Delanian and Lefaix, 2004).

Controversy exists over the management of ORN with conservative measures (antibiotics, analgesics, oral hygiene) suggested for early or low grade ORN, and surgical management for advanced or refractory case (Rice et al., 2015).

Trismus

Trismus is defined as reduced mandible mobility leading to a maximal interincisal opening of 35 mm or less (Dijkstra et al., 2006). The limited mouth opening has a negative impact on quality of life as it can cause difficulties in chewing, speaking, delivering of proper dental care and cancer surveillance (Lee et al., 2015). Trismus in head and neck oncology is related to fibrotic damage to the muscle of mastication, mainly the pterygoid and the masseter muscle, and the temporomandibular joint (Dijkstra et al., 2004).

The incidence of trismus is declined from 25.4% to 5% following the introduction of IMRT regimen, whereas it does not seem to be affected by altered-fractionated radiotherapy (Bensadoun et al., 2010).

Indeed a recent multicentre randomised study (ARTSCAN) comparing conventional versus accelerated RT in 124 patients with HNC failed to find any significant difference in patient-reported trismus or maximal interincisal distance between the two trial arms (Lindblom et al., 2014).

Different treatment modalities have been suggested. Physiotherapy using various external opening devices is generally considered the standard choice of treatment for trismus, although evidence of its effectiveness is not convincing (Scherpenhuizen et al., 2015).

Radiotherapy-associated xerostomia

Radiotherapy-associated xerostomia, the most common debilitating consequence of the radiation therapy for HNC, will be discussed in the next section (1.3).

1.3 Radiotherapy-associated xerostomia

1.3.1 Salivary gland anatomy

The human salivary gland system includes major and minor salivary glands. The paired parotid, submandibular, and sublingual are major salivary glands. Additionally, the mucosa of the upper aerodigestive tract is lined by hundreds of minor salivary glands.

The parotid glands are the largest of the major salivary glands and they are located in the preauricular region and along the posterior surface of the mandible. Each parotid gland is divided by the facial nerve into a superficial lobe and a deep lobe. The parotid duct, also known as Stensen's duct, secretes serous saliva into the vestibule of the oral cavity. The submandibular gland is the second largest major salivary gland and is located between the inferior edge of the mandible and the digastric muscle. The submandibular gland, as the sublingual gland, has both mucous and serous cells that empty into ductules, which in turn empty into the Wharton's duct. The smallest of the major salivary glands is the sublingual gland, which lies within the anterior floor of the mouth, superior to the mylohyoid muscle

and deep to the sublingual folds opposite the lingual frenulum. Several ducts (of Rivinus) from the superior portion of the sublingual gland secrete directly into the floor of mouth (Holsinger and Bui, 2007).

About 600 to 1,000 minor salivary glands, ranging in size from 1 to 5 mm, line the oral cavity and oropharynx producing a mucous secretion. The greatest number of these glands are in the lips, tongue, buccal mucosa, and palate, although they can also be found along the tonsils, supraglottis, and paranasal sinuses (Holsinger and Bui, 2007).

1.3.2 Salivary glands physiology

Salivary fluid is composed of more than 99% water and a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins (enzymes, immunoglobulins, antimicrobial factors, mucosal glycoproteins) (De Almeida et al., 2008).

Saliva has the important function of lubricating and protecting the oral cavity and upper pharynx, modulating the microbial population and providing stabilisation and remineralisation of teeth.

The salivary gland product plays a key role in the initial phase of the digestion of carbohydrates and fats through two main enzymes: ptyalin, an α-amylase able to cleave the internal α-1,4-glycosidic bonds of starches to yield maltose, maltotriose, and lingual lipase, a α-limit dextrin produced by lingual salivary glands to break down triglycerides (Pedersen et al., 2002).

Importantly the salivary fluid is involved in taste sensitivity, transporting the taste substances to the taste receptors (Motsuo, 2000).

Saliva aids speech by facilitating movements of the lips and tongue. Furthermore its mucous composition aids the lubrication of food particles during the act of chewing, which serves to mix the food with saliva. Lubrication eases the processes of swallowing and the progression of the bolus down to the oesophagus (Holsinger and Bui, 2007).

Saliva exerts several important actions in the maintenance of tooth and mucosal integrity. The antibacterial properties of saliva, mainly due to the presence of immunoglobulin A, lysozyme and lactoferrin allow inhibition of bacterial growth. Saliva also serves as a protective buffer for the teeth, reducing the rate of tooth demineralization due to the content of bicarbonate, phosphate and proteins (Pedersen et al., 2002).

A healthy adult produces at least 500 mL of saliva in 24 h (Porter et al., 2004).

The secretory unit of the salivary gland is composed of acinar cells, myoepithelial cells, intercalated duct, striated duct and excretory duct. Saliva produced from the acini is relatively isotonic to plasma but as the saliva is transported in the oral cavity through the ductal system, it becomes hypotonic to plasma, with high concentration of potassium and bicarbonate and low of sodium and chloride (Turner and Sugiya, 2002).

Saliva secretion is stimulated by both branches of the autonomic nervous system. Parasympathetic innervation of the major salivary glands leads to glandular vasodilation as well as myoepithelial cell contraction. The sympathetic innervation results in myoepithelial cell and changes in blood flow manifesting as vasoconstriction and vasodilation (Proctor and Carpenter, 2007).

The amounts of saliva supplied by the salivary glands changes from resting condition to stimulation. During unstimulated salivation, the majority of the saliva is produced by the submandibular glands, only 20% by the parotid, and approximately 5% by the sublingual glands, whereas during stimulation two thirds of secretion is from the parotid gland (Pedersen et al., 2002) (Figure 1-5).

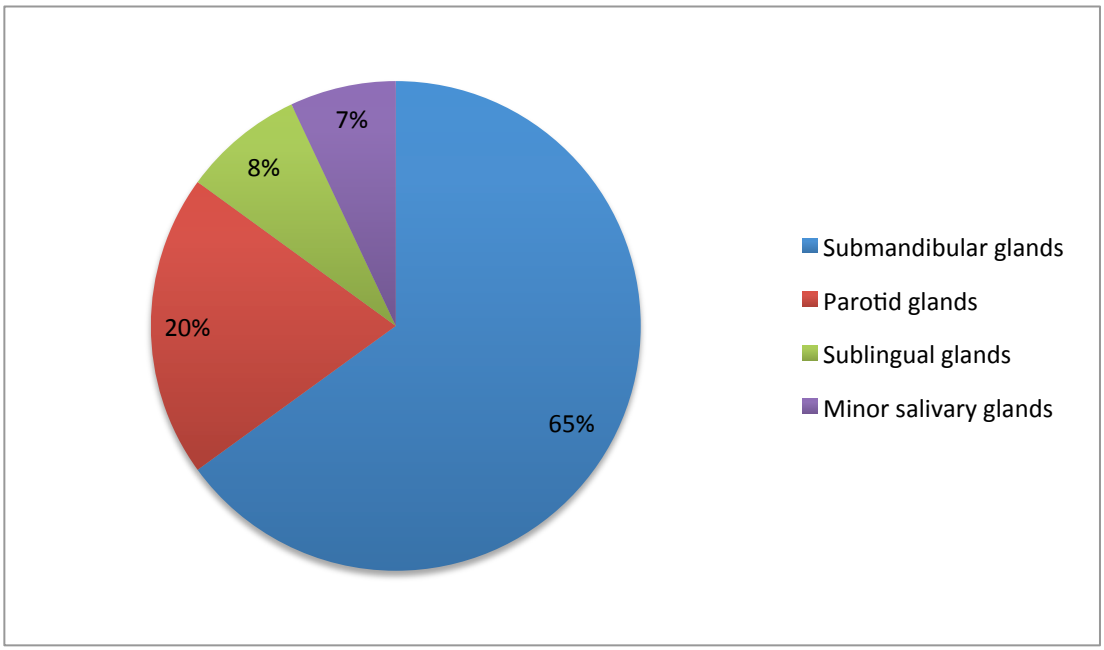


Figure 1-5 Secretory contributions of the salivary glands for resting salivary flow (Leo M. Sreebny, 2010)

1.3.3 Definition

Salivary gland hypofunction can be defined as an unstimulated whole saliva flow rate <0.1ml/min or stimulated whole saliva flow rate <0.7 ml/min (Streebny and Valdini, 1988, Ericsson and Hardwick, 1978). A reduction of salivary secretion can be associated with a subjective perception of oral dryness (xerostomia), which is estimated to occur when unstimulated flow rate is reduced by 45–50% (Dawes, 1987).

1.3.4 Prevalence of Radiotherapy-associated xerostomia

Xerostomia is the most common late side effect of RT to the head and neck region as the major salivary glands are usually within the field of treatment (Bjordal et al., 1994b, Harrison et al., 1997). The prevalence of RT-associated xerostomia varies with respect to RT field, dose, regimen, and technique (Table 1-2). Overall 63% to 93% of individuals exposed to head and neck RT develop xerostomia (Jensen et al., 2010b, Wijers et al., 2002).

Table 1-2 Prevalence of xerostomia by post-treatment phase and type of radiation therapy, adapted from Jensen et al. (2010b)

Type of Cancer Therapy	Baseline	During RT	1-3 months post RT	3-6 months post RT	6-12 months post RT	1-2 years post RT	>2 years post RT
2D-RT	10.4%	81.4%	70.9%	83.2%	71.5%	83.8%	90.9%
3D-RT	0%	NR	46.7%	74.5%	90.3%	75.4%	69.4%
IMRT	11.8%	100%	89.4%	72.7%	90.1%	66 %	68.1%

As already mentioned the introduction of new radiotherapy modalities has allowed a selective sparing of parotid gland tissue and a reduced prevalence, as well of severity, of xerostomia. Patients undergoing IMRT have a 24% risk reduction of developing clinician-rated xerostomia (RTOG) (HR 0.76; CI 0.66-0.87) compared to those treated with 2D-RT or 3D-RT. This benefit was statistically significant from 6 months to 5 years after completing the cancer treatment (Marta et al., 2014).

1.3.5 Pathophysiology

The mechanism of RT-associated salivary damage is not fully understood and the apparent radiosensitivity of the salivary glands is still an enigma. Their nature of highly differentiated cells with slow turnover would exclude their radiosensitivity. On the contrary, changes in quantity and composition of saliva occur also immediately after RT, indicating that the salivary glands are

highly sensitive to radiation (Burlage et al., 2001). Radiation induces a decline in amylase activity, bicarbonate and pH level, and a significant increase in osmolarity and viscosity. Furthermore alterations in the concentration of sodium, chloride and calcium, mucin 5b and mucin C7 have been observed (Randall et al., 2013).

Radiation-associated xerostomia and hyposalivation typically start early during cancer treatment: during the first week of radiotherapy a 50–60% decrease in salivary flow can be observed; after 7 weeks of conventional radiotherapy salivary flow can diminish to approximately 20% of the baseline value (Dirix et al., 2006).

Several studies have assessed the dose-response relationship between cumulative radiation dose and salivary gland damage. As previously mentioned following a cumulative exposure of >40 Gy, severe salivary gland damage (>75% of the baseline value) can be expected (Deasy et al., 2010).

Based on animal experiments, salivary glands damage induced by radiotherapy can be divided in three main phases (Konings et al., 2005).

In the first phase (0-10 days) water excretion is impaired with no cell loss; amylase and other protein secretions are unaffected. Clinically saliva becomes more dense and viscous. The second phase (10-120 days) is characterised by structural damage to the plasma membrane of the acinar cells with reduction in amylase secretion. The third phase (120-240 days) represents the late radiation damage, with loss of functional acinar cells as well as progenitor and stem cells. Histological observations suggest that some regeneration of acinar cells does take place after irradiation, yet the newly formed cells are functionally impaired due to the presence of radiation-

induced damage to ducts, blood vessels and nerves, as well as replacement of parenchyma with fibrotic tissue (Figure 1-6).

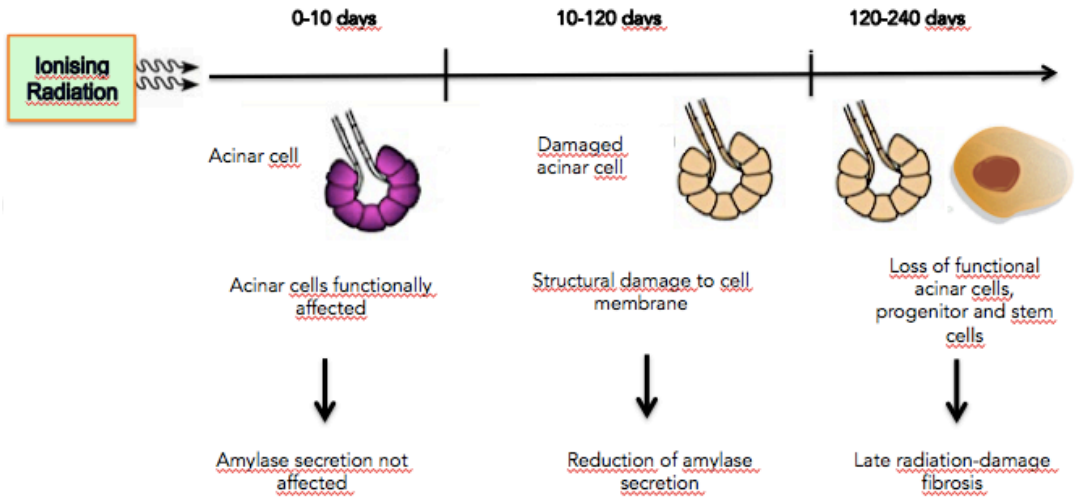


Figure 1-6 Illustration of the phases of salivary glands damage associated by radiotherapy

Two separate mechanisms seem to cause damage to salivary glands: the first mechanism involves selective membrane damage resulting in altered intracellular signal transduction and defects in cellular functioning. The second mechanism includes the loss of secretory cells as a result of progenitor cell death (Konings et al., 2005).

Eisbruch et al (2001) demonstrated the development of cellular degranulation and degeneration as soon as one hour after delivery of a single radiation dose of 2.5 Gy, whereas at 6 hours there was evidence of serous cell necrosis. Exposure to higher radiation dose was associated with degenerative changes progressing over time, atrophy and fibrosis. In addition to the necrosis, ionizing radiation can also activate apoptosis. Apoptosis culminates in the rapid clearance of cells by phagocytosis without inflammation and could be theoretically prevented by pre-treating the parotid

acinar cells with an agent capable of inhibiting radiation-associated apoptosis (Guchelaar et al., 1997). The relevance of radiation-induced apoptosis to the overall physiological function of the salivary gland, however, has not yet been elucidated, with some studies in rats showing how apoptosis could not explain the significant loss of gland function (Paardekooper et al., 1998). In contrast, studies in mice showed that radiation-induced apoptosis is dose dependent in parotid glands (Humphries et al., 2006).

Salivary gland dysfunction in irradiated patients is considered irreversible although a partial recovery of salivary function 12-to-18 months after treatment completion can take place (de la Cal et al., 2012). Little is known about the changes of salivary gland function with time, as the majority of the studies had a maximal follow-up of 2 years.

1.3.6 Symptoms and clinical manifestation

The insufficient wetting and lubrication of the oral mucosa observed in individuals with RT-associated salivary gland hypofunction can cause significant difficulties with speaking (dysarthria), taste (dysgeusia), swallowing (dysphagia), and mastication. The inhibition of taste sensation and the impairment of chewing and swallowing can affect patients' nutritional status. Patients can also experience intra-oral burning sensation and high sensitivity to salty and spicy foods. Wearing dentures may be extremely uncomfortable due to the reduced surface tension between the dry mucosa and the prosthesis (Hammerlid and Taft, 2001). Symptoms and signs of radiotherapy-induced xerostomia are summarised in Table 1-3 and Table 1-4.

Table 1-3 Symptoms of radiotherapy-associated xerostomia

General problems	Oral discomfort Lip discomfort Cracking of lips
Eating-related problems	Taste disturbance Difficulty chewing Difficulty swallowing
Speech-related problems	Difficulty speaking
Oral hygiene	Halitosis
Dental problems	Poorly fitting dentures leading to trauma of oral mucosa
Psychosocial problems	Embarrassment Anxiety Depression Social isolation
Miscellaneous problems	Sleep disturbance

On examination the lips of individuals with xerostomia can appear desquamated and fissured, and the oral mucosa atrophic, pale, and hyperaemic. Patients with xerostomia have an increased risk of oral infections, including candidal infection and sialadenitis (Abendstein et al., 2005, Jensen et al., 2010b, Wijers et al., 2002). Dental caries are common and can be severe and difficult to manage.

Table 1-4 Signs of radiotherapy-associated xerostomia

Angular cheilitis	Thick saliva
Salivary gland enlargement	Glazed, dry mucosa that tend to form wrinkles
Lobulated partial/complete depapillated tongue	Candida infection
Gross accumulation of plaque	Dental caries, including cervical caries

1.3.7 Diagnosis and Assessment of xerostomia

Diagnosis of xerostomia and salivary gland hypofunction relies on a detailed history and clinical examination. A number of patient-reported and clinician-rated outcome measures have been introduced to assess the severity of this condition.

Three patient-reported outcome measures have been used in clinical trials focusing on radiotherapy-associated xerostomia: the Visual Analogue Scale, the University of Michigan Xerostomia Questionnaire and the Xerostomia

Inventory. The Visual Analogue Scale (VAS) is the most used patient-reported outcome measured, being brief and freely available. Originally designed to assess pain, it is a self-assessment tool consisting of a 10-cm horizontal scale to indicate the severity of oral dryness (Pai et al., 2001). The University of Michigan Xerostomia Questionnaire was developed and validated by Eisbruch in 2001 (Eisbruch et al., 2001). The questionnaire consists of 8 questions addressing problems in communicating, eating, and sleeping functions. All questions are rated on a Likert scale to produce a final summary score between 0 and 100. A Xerostomia Questionnaire scores ≤ 30 corresponds to mild to no symptoms of xerostomia, with higher scores representing more severe xerostomia symptoms. The Xerostomia Inventory, has been developed and validated in 1999 by Thomson et al. (Thomson et al., 1999). The XI consists of 11-item summated rating scale with response options being “Never” (scoring 1); “Hardly ever” (2); “Occasionally” (3); “Fairly often” (4); or “Very often” (5). Each individual's responses are scored and summed to give a single XI score, which has a theoretical range from 11 to 55, with higher scores representing worse symptoms and a score of 14.5 or lower considered normal.

A total of five clinician-rated outcome measures have been used in clinical trials on radiotherapy-associated hypofunction, the most used being the quantitative measurement of salivary output (sialometry).

The RTOG scoring criteria defines acute radiation effects to salivary glands (within 3 months of the commencement of therapy) and chronic radiation effects, with scores ranging from 0 to 5 (0 = none; 1 = slight dryness of the mouth, good response on stimulation; 2 = moderate dryness, poor response

on stimulation; and 3 = complete dryness, no response on stimulation; 4= fibrosis; 5= death) (Cox et al., 1995). The two large organisations that initiated several multicentre clinical trials in Europe and North America, the EORTC and the RTOG, formed a working group to update and improve the system for recording late injuries to normal tissues. The Late Effects of Normal Tissues (LENT)–Subjective, Objective, Management, and Analytic (SOMA) is the resulting system and has been in use since 1995 (Pavy et al., 1995). The LENT-SOMA scoring system of xerostomia includes subjective grading consisting of an evaluation of dryness and objective findings of mucosal moisture. In 2003 the National Cancer Institute published the Common Terminology Criteria for Adverse Events (CTCAE) which incorporated the LENT SOMA items with early and late effects contained in one system, and it is now widely accepted throughout the oncology community as the standard severity grading scale for adverse events in cancer therapy clinical trials (Baldwin, 2003).

Three clinical trials have used a Modified Schirmer's Test, adapted from the test used routinely by ophthalmologists to measure the tear film wetness (Kohler and Winter, 1985, Davis and Marks, 1986, López-Jornet et al., 1996). One clinical trial used the Oral Assessment Guide (Andersson, 1999), consisting of eight categories: voice, swallow, lips, tongue, saliva, mucous membranes, gingival and teeth or dentures. Each category is rated by a scale of 1, 2 and 3, from normal findings (1) to severe (3). A sum score from the eight categories is calculated giving a range between 8, normal findings, and 24, severe alterations.

The vast majority of clinical trials used the salivary gland function output measurement (sialometry), which is also used in clinical setting as objective measure of hyposalivation (Dutta et al., 1973). Salivary gland function is assessed at rest and upon stimulation by measuring the whole-mouth saliva, or by selective suction from the glands' canals orifices. The methods involve the collection of saliva over a period of time (usually for at least 5 min), the collected saliva is weighed, the volume determined assuming a specific gravity of 1.0, and the flow rate (mL/min) is reported. Stimulation is typically done by application to the dorsum of the tongue of a chemical (e.g. 2% citric acid) or mechanical (e.g. chewing) stimulation. Several methods have been traditionally used to collect and measure whole mouth saliva. Saliva is either allowed to drain into a receptacle for weight or volume determinations (draining method), or is collected in the oral cavity and then voided into a receptacle (spitting method). A suction tube can be used to draw saliva from the floor of the mouth into a vessel (suction method), or pre-weighted cotton swabs are inserted to absorb saliva collecting on the floor of the mouth (swab method). Navazesh et al. (Navazesh and Christensen, 1982) compared the four assessment methods among 17 subjects. The draining method was recognized as cumbersome, procedurally distasteful, and prohibitively time-consuming for use in large epidemiologic studies. The study also found the swab method to be unreliable, whereas the suction method had the highest test/ retest reliability, but consistently yielded more saliva volume than the spit or draining methods. The authors thus concluded that the spitting method was preferable in terms of reliability and simplicity. There is no satisfactory method for selective measurement of the minor salivary glands function after radiation. No thorough comparison has been made of the reliability, validity,

and inter-correlation among the various salivary collection procedures, nor there has been a comprehensive comparison between resting and stimulated patterns of flow rate.

Sialometry has been used in nearly twenty clinical trials but significant discrepancies in the methods of performing sialometry can be observed, with time of collection ranging from 1 minute to 10 minutes and a number of different techniques used to assess the salivary flow rate. The most common technique is to assess the whole unstimulated and stimulated flow rate by spitting methods, although used techniques included unstimulated and stimulated parotid salivary flow, only unstimulated salivary flow and unstimulated and stimulated sublingual/submandibular salivary flow.

As mentioned in the definition of xerostomia, salivary hypofunction is commonly defined as unstimulated whole saliva flow rates of <0.1 mL/min or stimulated whole saliva flow rate of <0.7 mL/min (Streebny and Valdini, 1988, Ericsson and Hardwick, 1978). The correlation between salivary flow rate and subjective oral dryness sensation, however, is weak (Diogo Löfgren et al., 2012).

Imaging techniques such as magnetic resonance imaging (MRI), ultrasonography, and computed tomography can identify post-radiotherapy morphological changes as indirect evidence of salivary gland hypofunction. Magnetic resonance can show RT-changes in the internal architecture, with hyperintense signals due the oedema related to the damage of blood and lymph vessels. However this technique was found not suitable for the chronic observation of gland hypofunction as it cannot predict the severity of radiation-associated xerostomia (Wada et al., 2009). Ultrasonography can

show hyperechoic areas in the parotid glands indicating patches of inflammatory infiltrate (Ying et al., 2007).

Functional changes can also be assessed and measured via scintigraphy with technetium-sodium pertechnetate, as damaged salivary glands fail to excrete the marker in the saliva, leading to its accumulation within the gland. This technique is minimally invasive and well tolerated by patients although it is not specific enough to distinguish between Sjogren's syndrome and radiation damage (Hermann et al., 1999).

Computer tomography has been widely used in head and neck cancer imaging but the information regarding its application in the post-radiotherapy salivary gland evaluation are very limited to justify its use (Cheng et al., 2011).

1.3.8 Impact of xerostomia on Quality of Life

Despite the psychological and social impact of xerostomia, few studies have focused their attention on the extent of its influence on quality of life.

Pow et al in a study from 2006 already described in the previous section, revealed a significant correlation between stimulated whole salivary flow and global health status and emotional function assessed through the EORTC QLQ-C30 questionnaire (Pow et al., 2006). Significant correlations were found between role function and stimulated parotid saliva flow. For the H&N35 measure, significant negative correlations were found between speech problem, xerostomia, sticky saliva, and stimulated parotid saliva flow rate.

A well-designed longitudinal study conducted by Jellema in 2007 indicated that xerostomia (assessed though RTOG) has a significant effect on different dimensions of QoL (EORTC QLC-C30), notably on overall QoL outcome and all functioning scale but emotional functioning (Jellema et al., 2007).

A longitudinal prospective study assessed the severity of xerostomia in relation to the QoL in 63 head and neck cancer patients (Kakoei et al., 2012). Although the follow-up of this study was limited at 6 weeks after radiotherapy and the study is biased by the choice of improperly validated outcome measures, it is interesting to note that, according to regression analyses, with each millilitre decrease in saliva secretion, the QoL score decreased of 2.25%. Conversely with a 1-point increase in the xerostomia score, there was a 1.65% decrease in the mean QoL score (Kakoei et al., 2012), with no significant changes to salivary flow rate.

A prospective clinical study, confirming the hypothesis that sparing the parotid glands would result in significant objective and subjective improvement of xerostomia in patients with HNC, also observed that QOL questions on eating/speaking function were significantly correlated with stimulated and unstimulated saliva flow at 6 months. Unfortunately it is unclear which QoL outcome measures was used in the study and so the generalizability of the study remains unclear (Chao et al., 2001).

Results were in agreement with the study performed by Lin et al in 36 head-and-neck cancer patients receiving IMRT. The relationship between the XQ (University of Michigan Xerostomia Questionnaire) and QoL questionnaire (previously validated) scores revealed no statistically significant correlation before RT, with a significant linear correlation at each of the post-RT

endpoint (3, 6 and 12 months) between xerostomia and the eating, emotional and pain domain of the QoL questionnaire (Lin et al., 2003).

1.3.9 Preventive strategies

There is controversial evidence regarding the protection of salivary glands parenchyma from radiation damage.

Pilocarpine

Pilocarpine is a cholinergic agonist that can stimulate exocrine glands by acting as a muscarinic agonist. Pilocarpine eye drops have been used for many years to treat glaucoma. In 1994, an oral formulation of pilocarpine was approved by the FDA for the treatment of radiotherapy-associated xerostomia, but one year before the approval, its use concurrent with radiation had been tested. The mechanism of action of concomitant pilocarpine is not fully understood. Animal studies have suggested that the protective effect of pilocarpine on salivary glands is due to the depletion of secretory granules in serous cells. It has been speculated that by depleting heavy metals such as Zn, Mn, and Fe found in secretory granules, pilocarpine can decrease radiation-associated lipid peroxidation of lysosomal membranes and subsequent serous cell autolysis (Atri et al., 2014).

There remain doubts on the mechanisms of action and no evidence to suggest efficacy of pilocarpine in preventing xerostomia and hyposalivation associated with RT.

Seven studies have investigated the protective effect of pilocarpine.

Zimmerman et al in a retrospective study suggested that the use of pilocarpine per day (5 mg 4 times a day) during radiation treatment and for 3

months after treatment completion can reduce damage to salivary gland (Zimmerman et al., 1997). Although the authors' conclusion is that the seventeen patients using pilocarpine had significant less xerostomia symptoms than the eighteen who did not use the medication, no clear data are presented in the paper and limited evidence of pilocarpine efficacy in preventing radiotherapy-associated salivary gland damage is provided by this study. A small double-blind randomised trial of nine patients has been performed by Valdez (Valdez et al., 1993). In the study the subjective outcome measure to assess xerostomia symptoms is unclear, as well as the results, however patients taking pilocarpine had smaller losses in stimulated functions compared to the placebo group, with no difference in unstimulated salivary flow. Early concomitant pilocarpine use during RT has been also evaluated by Nyarady (2006) in a prospective unblinded randomised study in which 66 head and neck cancer patients were randomised to receive 5 mg pilocarpine three times a day from the beginning of RT for 12 weeks or to receive the same dosage of pilocarpine at the end of RT for 6 weeks. At the end of the study xerostomia symptoms of patients taking pilocarpine during RT returned closer to baseline compared with the control group, and the first group had also an improved salivary flow. However, three double-blind randomised clinical trials, where 58, 170 and 130 patients were assigned to treatment with pilocarpine 5 mg five, four and three times daily for five, two and 4 weeks respectively, failed to replicate positive outcomes of prophylactic pilocarpine (Gornitsky et al., 2004, Burlage et al., 2008, Warde et al., 2002). Finally the last study is burdened by the high dropout rate (35%) (Haddad and Karimi, 2002).

Amifostine

Amifostine is a radioprotective agent currently approved for the prevention of radiation-associated xerostomia. Amifostine is dephosphorylated by alkaline phosphatase to the active cytoprotective thiol metabolite, WR-1065, which can act as a scavenger reducing the free radicals generated by radiation. It binds highly reactive nucleophiles preventing their reaction with DNA and relevant DNA damage. A study suggested that amifostine could also inhibit the apoptotic process, reducing radiation-associated cell death (Saavedra et al., 2010).

Several studies have investigated the efficacy of amifostine in preserving salivary glands function and reducing radiation-associated xerostomia, clarifying its potential adverse side effects. Intravenous amifostine can cause adverse side effects such as hypotension (62% of patients) and nausea, which seem to occur less frequently when amifostine is administered subcutaneously (Bardet et al., 2011). There is no evidence to suggest that amifostine may have a tumour-protective activity and reduce efficacy of anti-cancer therapy (Bourhis et al., 2011, Sasse et al., 2006).

The effectiveness of amifostine in preventing RT-associated salivary gland damage and xerostomia has been investigated in six randomised clinical trials, which have been pooled together in a recent systematic review and meta-analysis (Gu et al., 2014). This meta-analysis clearly highlighted the efficacy of amifostine in reducing the risk of developing acute and late xerostomia by 40% respect to placebo or controls (observation), without tumour protection. According to this study, however, patients receiving concomitant chemoradiation did not benefit from the risk reduction. The reduction of radiotherapy-associated toxicities showed in clinical trials, which

also include dysphagia and mucositis, should be weighed against the toxicity associated to the amifostine itself. The other aspects to consider are the costs of the therapy and logistic problems, as there is the need to administer the drug immediately prior to each RT session. Subcutaneous amifostine administration was not found to be superior to intravenous administration in terms of patient compliance or efficacy (Kałuzny et al., 2014).

Submandibular glands transfer

In an effort to prevent radiation-associated xerostomia Jha and Seiklay firstly reported on a surgical procedure to transfer the submandibular gland out of the main RT field (to the submental space). This approach can be performed during cancer resective surgery, with an impact of additional 45 minutes to the duration of surgery. Jha et al (2003) yielded encouraging results with this technique in a prospective phase II trial: 81% of the 43 patients who had salivary gland transfer reported to have none or minimal xerostomia at the end of RT and 19% reported moderate to severe xerostomia. Two randomised trial have been conducted to investigate the clinical efficacy of this techniques to prevent xerostomia after radiotherapy. Zhang randomised 65 patients with nasopharyngeal carcinoma to either unilateral submandibular gland transfer before RT or no intervention. The study reported a higher salivary flow rate up to 60 months after radiotherapy completion and a significant higher proportion of patients experiencing none or mild clinician-rated xerostomia (RTOG) in the experimental group (Zhang et al., 2014).

Submandibular salivary gland transfer has also been compared to treatment with pilocarpine during and 3 months after radiotherapy in a multicentre

phase III randomised trial recruiting 120 participants (Jha et al., 2009). At 6 months patients in the gland transfer arm showed improvement in salivary flow and in the domain “amount of saliva” of the University of Washington Quality of Life questionnaire compared to the pilocarpine group (50% vs. 26%). No additional subjective or objective assessments have been performed in the study. Surgical complication associated with this technique include facial oedema (13.6%), submental swelling (19%), shoulder weakness (4.5%) and neck numbness (6.8%) (Jha et al., 2012).

Acupuncture

Acupuncture has been suggested to induce physiological effect, modulate neurological processes within the spinal cord gating mechanisms, the cerebral subcortical nuclei and the hypothalamic-endocrine axis (Sagar, 2008), as well as stimulate the autonomic nervous system, increase peripheral blood flow, and enhance levels of vasoactive intestinal polypeptide, which might in turn increase salivary flow rates (Dawidson et al., 1999, Dawidson et al., 1998).

A single-centre randomised feasibility trial conducted by Meng (Meng et al., 2012) showed that forty individuals receiving acupuncture concurrently to radiotherapy had significantly lower scores in a xerostomia questionnaire at 6 months and greater salivary flow rate with respect to the control group (observation). Further large-scale, multi-centre, placebo-controlled randomised trials are needed to confirm these encouraging preliminary findings.

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBOT) aims to counteract by oxygenation the effect of radiotherapy to surrounding normal tissues, which can become hypocellular, hypovascular, and hypoxic. HBOT has been traditionally used in head and neck oncology for the treatment and prophylaxis of radionecrosis of bone and soft tissue. During daily treatments, patients are placed in chambers that are pressurized to 2 to 3 atmospheres absolute and encourage to breathe 100% oxygen for a total of 20 to 40 dives (Fox et al., 2015). Two randomised clinical trials have tested the efficacy of HBOT in reducing radiotherapy side effects. Teguh in 2009 randomised 19 head and neck cancer patients to receive HBOT shortly after radiotherapy completion or no treatment (Teguh et al., 2009). However only 19 patients out of 132 of the sample size calculation were recruited due to a premature closure of the study. Clinician-rated outcome measure were not used in the study but patient-centred outcome measures showed that xerostomia was significantly less severe in the HBOT group 18 months after radiotherapy completion, the difference between group being 3 on a 0-10 VAS severity scale.

A recent systematic review has been published by Fox in 2015 (Fox et al., 2015) concluding that HBOT can induce long-term improvement in xerostomia. However the strength of these conclusions are limited by the number of studies included (n= 7), the design of included studies (five were prospective in nature but only two were randomised) and the research questions, with the majority of studies focusing on the treatment of osteonecrosis.

2 The management of radiotherapy- associated xerostomia

2.1 Systematic review and meta-analysis of the interventions for the management of radiotherapy-associated xerostomia

2.1.1 Introduction

A wide range of interventions for radiotherapy-induced salivary gland hypofunction are available (Plemons et al., 2014). Stimulation of salivary gland function may be appropriate for patients with some degree of residual salivary gland parenchyma, and it can be attempted through sialagogue medications (parasympathetic agonists such as pilocarpine and cevimeline) (Brimhall et al., 2013) or activating the salivary reflex arch via chewing gums or sucking pastilles and lozenges (Furness, 2011). Topical application of salivary substitutes (artificial saliva) can offer some benefit by providing a moisture-retaining coating onto the oral mucosa (Dost and Farah, 2013). Other interventions, such as acupuncture, have also been used to increase saliva production, possibly by enhancing peripheral blood flow (Wolff et al., 2012). However no clear guidance for clinicians is available as randomised trials of available treatment modalities have produced unclear results and there is currently little robust evidence to inform the management of hyposalivation and xerostomia in this population. Some of the available systematic reviews have not specifically focused on HNC patients but rather considered individuals with xerostomia due to a variety of causes (Furness, 2011). Others presented a number of methodological weaknesses (Lovelace et al., 2014, Jensen et al., 2010a, Mercadante et al., 2015). Overall, the effectiveness of available interventions in this population remains unclear, and it is perhaps not surprising that the majority of the HNC survivors report

to rely on drinking frequent sips of water (Cassolato and Turnbull, 2003) which does not increase salivation and is unlikely to overcome chronic xerostomia or prevent its complications. We have undertaken this systematic review and meta-analysis in order to estimate the effectiveness of available treatments and contribute to the development of guidelines for the management of radiotherapy-associated hyposalivation and xerostomia.

2.1.2 Methods

Study design

We developed a protocol that defined inclusion criteria, search strategy and outcomes of interest. The reporting of this systematic review and meta-analysis adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

Literature search

For the identification of studies to be included in this review, we developed detailed search strategies for each database (Medline, Embase, The Cochrane Central Register of Controlled Trials, Cinahl, Amed). We searched reference lists of retrieved reports and textbooks for additional references. The last literature search was performed on the 19th July 2015. Citations were screened and full reports of potentially relevant studies obtained. Study inclusion criteria were (i) design: randomized controlled trials; (ii) population: adults with diagnosis of xerostomia (xerostomia symptoms) following head and neck radiotherapy; (iii) intervention: techniques designed to stimulate saliva production (e.g. topical and systemic sialogogues, acupuncture, laser therapy, and electrostimulation) or to replace saliva (e.g. saliva substitutes); (iv) control group: placebo, no intervention, another active intervention or a combination of the aforementioned options. The interventions could be given

by any route, formulation, or dose. Studies had to contain sufficient clear information on the effect of the experimental treatment on clinical outcome to be included. No language restrictions were imposed.

Outcome measures and endpoints

The primary subjective outcome measure of this review was the mean overall change in xerostomia symptoms, which was assessed by change in a visual analogue scale (VAS). Secondary objective outcomes were changes in QoL and salivary flow. We looked in detail at the time endpoints used for collection of the outcome measures; in particular we considered whether measurements at endpoint were taken shortly after the intervention (e.g. few minutes or hours) or away from treatment completion (therefore representing perceived symptoms and salivary flow during resting condition).

Selection process and quality assessment

Titles and abstracts of the references were reviewed to exclude articles out of scope. Full-text articles of potentially relevant records were assessed for eligibility by two independent reviewers (VM, AH). Any disagreements between reviewers were resolved by discussion until consensus was reached. The methodological quality assessment of the selected trials was performed according to the Cochrane Collaboration tool for assessing risk of bias, documenting the method of sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias.

Data extraction

The following data were extracted by VM and AH: (i) study population; (ii) type, dosage, frequency and duration of intervention, (iii) control group; (iv)

xerostomia and hyposalivation outcome measures; and (v) effects on psychosocial outcomes (QoL).

Meta-analysis

We summarized the effect size for continuous data using the mean difference (MD) with 95% confidence intervals (95% CI). For categorical data, reported xerostomia relief and salivary flow changes were dichotomised into two categories (improvement or no improvement), and we calculated odd ratio (OR) of improvement, with 95% CI. A fixed effect model was used unless statistical heterogeneity was significant ($p<0{\cdot}05$), after which a random effect model was used. Point estimates of effect were statistically significant when the CI did not cross 1 for OR and 0 for MD.

Role of the funding source

There was no dedicated funding source for this study.

2.1.3 Included studies

Figure 2-1 shows the process of study selection, leading to the inclusion of 19 studies in the systematic review, with a total of 1710 patients. Ten of 19 included studies were designed as parallel group studies whereas nine studies had a crossover design with washout periods of one week on average. Six studies were conducted in the USA, three in the United Kingdom, two in Canada, one in China, Germany, Hungary, Korea, Netherlands, Sweden, Thailand and Brazil. Sixteen trials (84%) provided funding information. Eight trials were funded by the pharmaceutical industry, four trials received government funding, and four studies received university funding. Trials recruited between 12 and 286 participants. The mean age of study participants was 58 years (SD 7), and 495 (29%) of them were women. Five trials provided data for systemic parasympathetic sialogogues vs.

placebo (two on pilocarpine and three on cevimeline), two studies focussed on topical pilocarpine vs. systemic pilocarpine or salivary substitutes and placebo, three trials studied commercially available “mouthcare systems” containing a combination of artificial saliva gel and/or oral rinse and/or a spray and/or a toothpaste, three studies focussed on saliva substitutes vs. other saliva substitutes or placebo, one trial investigated hyperthermic humidification through a nasal cannula (Vapotherm) vs. a standard humidifier, three trials studied acupuncture vs. sham acupuncture or educational oral care sessions, one trial explored acupuncture-like transcutaneous nerve stimulation and one low-level laser therapy. In total eight trials used a placebo or sham intervention as comparator whereas in eleven studies controls were active interventions. The duration of the included trials varied from two weeks to 15 months (mean 8.9). Seventeen studies used changes in xerostomia symptoms as an outcome. At endpoint, symptoms were assessed shortly after administration of the intervention in four studies (Cho, 2008, Blom, 1996, Chambers et al., 2007) and after 180 minutes in one trial (Taweechaisupapong, 2006) and therefore refer to symptoms perceived by participants during enhancement of salivary gland function. Two studies collected xerostomia symptom measurements one or more weeks after completion of the experimental treatment (Simcock, 2013, Wong et al., 2015), therefore referring to symptoms perceived by patients during resting salivary condition. Timing of measurement collection at endpoint was unclear in twelve studies (LeVeque, 1993, Johnson, 1993, Witsell, 2012, Criswell, 2001, Epstein, 1999, Nagy, 2007, Shahdad, 2005, McMillan, 2006, Jellema, 2001, Momm, 2005, Davies, 1994, Saleh et al., 2014).

Twelve trials used changes in salivary function as an outcome. At endpoint, salivary function was assessed shortly after the intervention in four studies (Cho, 2008, Blom, 1996, Chambers et al., 2007) after 60 minutes in two studies (LeVeque, 1993, Johnson, 1993) and after 180 minutes in one study (Taweechaisupapong, 2006) and therefore refer to enhanced salivary gland flow. Two studies assessed salivary flow one or more weeks after completion of the experimental treatment (Simcock, 2013, Wong et al., 2015) therefore referring to resting salivary condition, whereas the timing of salivary flow collection at endpoint remained unclear in three studies (Nagy, 2007, Epstein, 1999, Saleh et al., 2014).

Five studies used changes in QoL scores as an outcome. Two studies collected QoL measures one or more weeks after completion of the intervention (Simcock, 2013, Wong et al., 2015) whereas timing of outcome collection was unclear in three studies (Saleh et al., 2014, McMillan, 2006, Witsell, 2012).

Risk of bias

We considered nine studies (47%) to have a low overall risk of bias (Figure 2-2). Adequate sequence generation and concealment was reported in 78% and 68% of studies respectively, which were therefore considered to be at low risk of selection bias. Blinding of participants to the allocated treatment by use of a placebo was done in 12 of the included studies (58%) and these trials were assessed as low risk of performance bias. Outcome assessors were blinded to treatment allocation in 13 trials (68%), which were considered to be at low risk of detection bias. Over 80% of the included studies reported complete outcome data without selective reporting.

2.1.4 Results of the systematic review

Systemic pilocarpine vs. placebo.

Two placebo-controlled trials with low risk of bias investigated the effectiveness of systemic pilocarpine in 146 and 134 patients respectively (LeVeque, 1993, Johnson, 1993). With respect to primary outcome of xerostomia symptoms reduction, both studies showed that 12-week use of 5 to 10mg oral pilocarpine three times a day gave rise to a reduction in xerostomia symptoms in significantly more patients (44% and 46%) than placebo (25% and 25%) (Johnson, 1993, LeVeque, 1993). Magnitude of improvement was unclear as both studies only reported the number of patients who had an arbitrary reduction of at least 25mm in the xerostomia visual analogue scale ("responders") without presenting absolute figures and with no indication whether this could represent minimally clinical significant difference. Also it remains unclear whether xerostomia symptoms at endpoint were assessed before or shortly after administration of pilocarpine tablets, and therefore whether they refer to symptoms perceived by patients when salivary function was in resting condition or shortly after treatment (that is acutely enhanced salivary flow). With respect to the secondary outcome of increased salivation, both studies showed that the use of a single 5mg pilocarpine tablet was associated with a short-term 60-minute increase in unstimulated whole salivary flow rate in significantly more patients (69.1% and 68.9%) than placebo (56.3% and 42.7%) (Johnson, 1993, LeVeque, 1993). The magnitude of improvement was however unclear as both studies only reported the number of patients who had any increase of salivary flow ("responders"). There was no information regarding the longer-term effects (i.e. beyond 60 minutes) of the intervention upon salivary flow rates and

clinical significance of the reported improvement remains unknown. Adverse side effects were seen more frequently in individuals using pilocarpine than in the placebo group and mainly consisted of sweating, urinary frequency and nausea. More patients in the pilocarpine group withdrew from the study because of adverse effects (15% vs. 10%).

Systemic cevimeline vs. placebo.

Two research groups assessed the effectiveness of oral cevimeline (30 mg three times daily) in three studies (Witsell, 2012, Chambers et al., 2007). Chambers et al reported two high quality clinical trials with low risk of bias, which enrolled 281 and 282 patients respectively and had similar design but different results. With respect to the primary outcome of reduction in xerostomia symptoms, one trial reported that 12 weeks cevimeline therapy was associated with an improvement in symptoms compared to pre-therapy in significantly more patients than placebo (48% vs. 33%; $P<0.01$). However, magnitude of improvement was unclear as the study only reported the number of patients who had any perceived improvement in the sensation of xerostomia ("better/much better") without presenting continuous outcome figures. The second trial, however, showed no significant difference. Of note, xerostomia symptoms were measured at endpoint shortly after administration of one tablet of 30mg cevimeline and therefore refer to symptoms perceived by patients during acute enhancement of salivary flow. With respect to the secondary outcome of increased salivation, both studies reported that 12-week use of cevimeline was associated with a significant, albeit small, increase in unstimulated whole salivary flow with respect to placebo (mean change from screening 0.044±0.099 mL/min and 0.052±0.161 mL/min; $P<0.01$). It is not known whether this increase in salivary flow was meaningful

to patients. Of note, salivary flow at endpoint was measured shortly after administration of one tablet of 30mg cevimeline and therefore refers to acutely enhanced salivary flow. Adverse side effects were seen more frequently in individuals using cevimeline and mainly consisted of increased sweating and gastrointestinal symptoms (dyspepsia). Intensity was usually mild-to-moderate but more patients in the cevimeline group withdrew from the study due to adverse effects were uncommon (~13-14% vs. 3-5%).

A second research group assessed the effects of 30mg cevimeline three times a day upon QoL, observing no significant differences between experimental and placebo groups after 6 weeks of treatment (Witsell, 2012). This study was considered at unclear risk of bias.

Systemic pilocarpine vs. "topical" pilocarpine vs. topical placebo.

Topical administration of pilocarpine has been attempted in order to minimize absorption and related toxicity. Taweechaisupapong et al reported the results of a cross-over placebo-controlled trial, which they claim was double-blinded, of 33 head and neck cancer patients who were randomly assigned to receive every ten days one single 5mg pilocarpine tablet (to be swallowed), or one pilocarpine lozenge (3 mg or 5 mg dose) or one placebo lozenge both to be dissolved in the mouth (Taweechaisupapong, 2006). With respect to the subjective outcome of reduced dryness symptoms, the study reported that the use of one 5mg lozenge was associated with a short-term (measured at 180 minutes) reduction in xerostomia in significantly more patients than the 3mg lozenge, 5mg pilocarpine tablet and placebo (69.7% vs. 63.6% vs. 12.1% vs. 42.4% respectively; $P<0.05$). However, the magnitude of improvement was unclear as the study only reported the number of patients who had an arbitrary reduction of at least 20mm in the xerostomia visual

analogue scale (“responders”). Clinical significance of the perceived improvement remains unknown. With respect to salivary flow, the topical use of one 3mg and one 5mg pilocarpine lozenge, as well as one 5mg tablet was associated with a significant short-term (measured within 180min) increase in unstimulated salivary flow compared to placebo. Again, the magnitude of improvement was unclear, as salivary flow rates were not reported. Of note, it seems that no significant difference between the use of pilocarpine lozenges (topical use) and tablet (systemic use) was observed. No adverse effects were reported. This study was considered at high risk of bias because it was not entirely double-blinded: investigators knew which patients were on pilocarpine tablets; similarly patients knew that one treatment was different from the other three. Also, it is rather questionable that dissolving pilocarpine lozenges in the mouth without spitting the content out (therefore swallowing the medication) really represents topical treatment with minimal/negligible systemic absorption.

Saliva substitutes vs. “topical” pilocarpine.

One cross-over randomised trial studied the effect of 3-months use of a spray containing mucin-based artificial saliva (Saliva Orthana) compared to a mouthwash containing pilocarpine (5 mg three times a day) (Davies, 1994). The study failed to demonstrate statistically significant difference in xerostomia symptom improvement between the two groups as recorded on visual analogue scale. This study has been classified as being at high risk of bias for unclear information upon blinding of study participants and investigators. Also, it is rather questionable that rinsing the mouth with pilocarpine mouthwash without spitting the content out (therefore swallowing

the medication) really represents topical treatment with minimal/negligible systemic absorption.

Mouthcare system vs. other mouthcare system.

Three trials evaluated the effects of commercially available "mouthcare systems" containing a combination of artificial saliva gel, and/or oral rinse, and/or spray, and/or a toothpaste (Epstein, 1999, Nagy, 2007, Shahdad, 2005). Epstein et reported in a high-quality cross-over trial that after 2 weeks of treatment patients using Biotene gel and toothpaste (enzyme and hydroxyethylcellulose-based artificial saliva) had a significantly reduced xerostomia symptom on waking (VAS change) and increased stimulated salivary flow compared to controls using carboxymethylcellulose gel and commercial toothpaste, when data were analysed for carryover effect. However the magnitude of the effect and timing of outcome measurements were unclear (Epstein, 1999). Nagy et al reported another crossover trial of 4 weeks of treatment with Biotene gel and toothpaste vs. carboxymethylcellulose gel and commercial toothpaste. They observed a significantly higher reduction in xerostomia symptoms and increase in unstimulated salivary flow in participants using Biotene system with respect to controls (mean change in VAS score of 3 for test vs. 0.7 cm for control, $P<0.01$; mean change in unstimulated salivary flow of 0.13 mL/5min for test vs. 0.0 in controls; $P=0.6$) (Nagy, 2007). The clinical meaningfulness of the reported changes is unknown. Of note, this study should be considered at high risk of bias: although study measurements refer to the post-radiotherapy period, the experimental intervention and placebo treatment were commenced during radiotherapy and therefore baseline measurements (week 0) do not really represent pre-study status. Shahdad et al studied the

effects of 2 weeks of therapy with Oral Balance or BioXtra mouthcare systems (gel, mouthwash and toothpaste) in a double-blind cross-over high-quality trial (Shahdad, 2005). Oral Balance and BioXtra are both enzyme-based salivary substitutes but the latter has higher viscosity and contains additional salivary peptides and immunoglobulins when compared with the former. Participants using BioXtra treatment achieved a greater improvement in xerostomia symptoms compared to those using Oral Balance mouth care system (mean difference in VAS score of 28.4 vs. 17.2 mm; $P<0.05$). Clinical meaningfulness of the reported effect size is unknown. This study was considered to be at low risk of bias.

Salivary substitutes vs. other salivary substitutes or placebo.

Three randomised crossover trials compared salivary substitutes vs. other salivary substitutes or placebo (Jellema, 2001, Momm, 2005, McMillan, 2006). McMillan experimented a novel intra-oral device for the slow release of Oral Balance gel versus an oral bolus of the same gel in a randomized single-blind crossover clinical trial. The use of the slow-release gel device for 4 weeks did not lead to a significantly different effect upon xerostomia symptoms (Xerostomia Inventory) or salivary flow with respect to the oral bolus of gel (McMillan, 2006). Changes in oral health-related quality of life scores (GOHAI) were higher in participants using the device (mean change was 1.38 vs. -2.1; $P=0.026$). Timing of outcome collection at endpoint was also unclear. We considered this study as being at high risk of bias because of its single-blind design.

Jellema assessed the effects of 1-week use of a xanthan gum-based salivary substitute (Xialine) versus placebo in double blind crossover trial of 30 participants and found no significant difference upon xerostomia symptoms

(xerostomia questions of QLQ-H&N35) (Jellema, 2001). Momm tested the effects of 1-week use of four different salivary substitutes (aloe vera gel, carboxymethylcellulose spray, rape oil, mucin spray) and reported a significant reduction in xerostomia symptoms with respect to baseline for all groups but not different among the 4 arms (mean xerostomia change from baseline 0.7-0.8 for all saliva substitutes; $P<0.05$) (Momm, 2005). Effect size was correctly reported but its clinical significance is unknown. Also timing of outcome collection at endpoint was unclear. We considered the studies of Jellema and Momm to be at high risk of bias due to the lack of information on blinding of participants and investigators.

Acupuncture vs. sham acupuncture or educational oral care sessions.

Three randomized controlled trials investigated the therapeutic efficacy of acupuncture in radiation-associated xerostomia (Cho, 2008, Blom, 1996, Simcock, 2013).

The single-blinded study of Cho et al reported no statistically significant difference in salivary flow or xerostomia symptoms changes in cases (real acupuncture) versus controls (sham acupuncture) after 6 weeks of twice-a-week treatment (Cho, 2008). Simcock et al studied the effects of 8 weeks of once a week acupuncture versus educational oral care sessions (Simcock, 2013). They observed that those having acupuncture were more likely to report any improvement in xerostomia symptoms (as per QLQ-H&N35 and other xerostomia questions) compared to controls (OR: 2.01; $P=0.03$) (Simcock, 2013). Magnitude of the effect was unclear, as well as clinical significance. There was no difference in salivary flow or quality of life score changes between groups. Of note, endpoint assessment was performed a

week after the completion of treatment and therefore refers to resting salivary flow. Blom et al studied the effects of 12 weeks of twice-a-week acupuncture versus superficial acupuncture in a randomised trial where the outcome assessor and the participants were blinded to the treatment. No statistically significant difference in salivary flow rates between the two arms was reported. Of note, no subjective xerostomia measures were taken (Blom, 1996). We deemed the three studies to have high risk of bias due to the overall poor reporting of randomisation and blinding.

Hyperthermic, supersaturated humidification vs. standard bedside humidifier.

One study assessed the effectiveness of a new device delivering hyperthermic, supersaturated humidification through a nasal cannula compared to a standard bedside humidifier in a randomized, controlled, crossover study (Criswell, 2001). The xerostomia symptom questionnaire and visual analogue scores showed no significant difference between the two devices in lessening xerostomia symptoms after 2 weeks of use. This study was considered at unclear risk of sequence generation, allocation concealment and blinding bias.

Acupuncture-like transcutaneous electrical nerve stimulation (ALTENS) vs. pilocarpine.

One multicentre unblinded study randomised 148 patients to either 12 weeks of acupuncture-like transcutaneous electrical nerve stimulation (twice-weekly for a total of 24 sessions of 20-minutes each) or pilocarpine (5mg three times a day) for the equivalent 12 weeks period (Wong et al., 2015). Outcomes were assessed at endpoint (9 months after randomisation) and revealed no significant difference in Xerostomia-related Quality of Life Scale score (XeQOLS) and salivary flow changes between groups. Of note, endpoint

assessment was performed several weeks after the completion of treatment and therefore refers to resting salivary flow. This study was considered at high risk of sequence generation, allocation concealment and blinding bias.

Low-level laser therapy vs. sham low-level laser therapy.

One randomised trial tested the effectiveness of low-level laser therapy in improving xerostomia symptoms (assessed through VAS), salivary flow and quality of life (assessed through the Oral Health Impact Profile questionnaire) in 23 patients (Saleh et al., 2014). The test group underwent two weekly sessions of low-level laser therapy for 6 weeks whereas the control group underwent the same number of sessions but a plastic tip blocked the emission of radiation. Endpoint assessments failed to show any significant difference between the groups with regards to long-term changes in xerostomia VAS, salivary flow rates or QoL scores from baseline. Of note, measurements at endpoints were taken shortly after the administration of the intervention and therefore refer to flow rate and symptoms perceived during enhancement of salivary function. Additionally it is unclear whether at least the patients or the outcome assessor were blinded to the intervention. This study was considered at unclear risk of sequence generation, allocation concealment and blinding bias.

2.1.5 Results of the meta-analysis

Four trials provided sufficient data to evaluate the primary outcome of the mean overall change in subjective xerostomia symptoms. Six studies allowed statistical analysis of the objective assessment of changes in salivary flow rate. It was not possible to analyse the QoL outcomes due to the lack of homogeneity between study measures. In relation to the primary outcome of reduction in xerostomia symptoms two comparisons were sufficiently

clinically homogenous to perform statistical pooling: systemic pilocarpine vs. placebo and systemic cevimeline vs. placebo (Figure 2-3). Two studies, with a total of 280 participants showed that the patients using pilocarpine for 12 weeks were more likely to have a 25mm or higher reduction in xerostomia VAS score compared to placebo (OR of 2.37, 95% CI 1.43-3.94). Two homogeneous studies with a pooled total of 563 participants showed that the patients using cevimeline for 12 weeks were more likely to report an improvement (better/much better) in the sensation of xerostomia compared to placebo (OR 1.37, 95% CI 0.98-1.91).

In relation to the secondary outcome we were able to compare the effect of acupuncture vs. sham/superficial acupuncture, cevimeline vs. placebo, and pilocarpine vs. placebo on unstimulated salivary flow rates (Figure 2-4 and 2-5). Two studies of acupuncture (6 and 12 weeks) versus sham/superficial acupuncture with a pooled total of 50 participants showed no increase (MD 0.00; 95% CI -0.02-0.03) in unstimulated salivary flow rate on continuous outcome measures. Pooled analysis of two RCTs of cevimeline versus placebo (total number of patients = 563) showed a small (MD 0.04, 95% CI 0.02-0.06) increase in unstimulated saliva flow rate in participants using cevimeline for 12 weeks with respect to controls. Two RCTs (total number of patients = 280) showed that the use of one tablet of pilocarpine is more likely to be associated with a short-term increase (60 minutes) in unstimulated salivary flow rate compared to one tablet of placebo (OR 2.27; 95% CI 1.37-3.76).

2.1.6 Discussion

No clear evidence-based guidance for clinicians is currently available to inform the management of radiotherapy-associated hyposalivation and xerostomia in HNC survivors. Available systematic reviews and meta-analyses on xerostomia management have not specifically focused on post-radiotherapy HNC setting (Furness, 2011) or present a number of methodological weaknesses (Lovelace et al., 2014, Jensen et al., 2010a). We have previously commented (Mercadante et al., 2015) on the questionable validity of the systematic review and meta-analysis of Lovelace et al, and the systematic review from Jensen et al also seem to have similar weaknesses such as the inclusion of non-randomised studies and linguistic constraints (Gregoire et al., 1995, Higgins et al., 2013). We have therefore undertaken this systematic review and meta-analysis in order to overcome limitations of previous studies and estimate the effectiveness of available treatments, so to contribute to develop evidence-based practice guidelines for the management of radiotherapy-associated hyposalivation and xerostomia in HNC survivors. Of note, we looked at a number of details of published trials that were not highlighted in previous reviews but we believe are important for the interpretation of the efficacy of interventions and therefore for clinical decisions upon patient management. These include the type of outcomes (xerostomia symptoms, salivary function, quality of life) and the timing of collecting outcome measurements at endpoint (shortly after administration of the intervention or away from treatment completion).

We identified 19 RCTs with a total of 1710 randomized patients, which qualified for inclusion. Meta-analyses were only possible for three of the interventions included in the systematic reviews because of the limited

number of studies and their heterogeneous designs including major differences in the comparator and outcome measures. Where data polling was possible, results of this review suggest that long-term use of systemic pilocarpine or cevimeline has a positive effect in reducing xerostomia sensation in HNC survivors after radiotherapy, with likelihood of improvement being higher for pilocarpine. However the effect size of such reduction was unclear for pilocarpine (at least 25mm on 0-100mm VAS) and not reported for cevimeline. It remains unknown whether a VAS change of at least 25mm is clinically relevant in this patient population, whereas the improvement in symptoms obtained with cevimeline do seem clinically meaningful (as patients reported to feel better/much better). Further, this positive effect seems to be limited to symptoms perceived shortly after the administration of the intervention, whilst no information is available with respect to xerostomia symptoms perceived under resting salivary conditions.

Pilocarpine and cevimeline also seem effective in increasing salivary flow. Our meta-analysis shows that long-term use of cevimeline can induce an increase in unstimulated salivation, though the relevant effect size seems small (MD of 0.04 mL/min). However patients reported to feel better/much better and therefore a MD of 0.04mL/min can be considered clinical meaningful. With respect to pilocarpine, available data only provide evidence of an increase in salivation after short-term use (one single tablet with salivary flow measured after 60 minutes), whereas the effect size, clinical significance, as well as the effects of long-term use are unknown. Again no information is available with respect to salivary gland function under resting conditions. With respect to acupuncture, data from our meta-analysis show

no evidence of increased salivary flow and unknown effect upon xerostomia symptoms due to lack of data.

There are practical implications of these results: clinicians managing HNC survivors with post-radiotherapy hyposalivation and xerostomia symptoms should consider prescribing long-term cevimeline therapy and expect that it will provide some reduction in xerostomia symptoms and a small increase in salivary flow which can be clinically meaningful, although likely to be short-lived. Similarly they should expect a reduction in xerostomia symptoms with long-term pilocarpine use, as well an increase in salivary flow after one tablet of pilocarpine, although these improvements are likely to have a short duration, unclear effect size, and unknown clinical significance. The toxicity of pilocarpine and cevimeline seems similar, possibly with a tendency for cevimeline to be better tolerated, although evidence is not robust as no direct comparison is available.

With respect to acupuncture, it seems difficult to support the clinical decisions of recommending this treatment modality to HNC survivors with radiotherapy-associated xerostomia as our meta-analysis suggests that it does not increase salivary flow in HNC cancer survivors after radiotherapy. It was not possible to merge data upon changes in xerostomia symptoms.

With regard to interventions not included in the meta-analysis, our systematic review of single studies suggests that there is no evidence, or very weak evidence, that “topical” pilocarpine, hyperthermic supersaturated humidification, acupuncture, ALTENS or lower-laser therapy can reduce xerostomia symptoms, increase salivary flow, or improve QoL in this patient population.

Salivary substitutes (gel and spray) and mouth care products (toothpaste and mouthwash) are widely used in the management of xerostomia (Jensen et al., 2010a) and accordingly our systematic review included six studies comparing the effects of salivary substitutes/mouth care products against other salivary substitutes/mouth care products or placebo. There was high heterogeneity among studies and therefore no data pooling was possible. Systematic review of single studies shows that there is no study but one comparing the effects of salivary substitutes vs. placebo. There is some weak evidence from 2 small studies, of which one is at high risk of bias, that enzyme-enriched mouth care products (gel and toothpaste) are superior to traditional carboxymethyl-cellulose gel and commercial toothpaste at reducing xerostomia symptoms and increase salivation. The effect size, where reported, seems to be moderate (23mm 0-100mm VAS) with unknown clinical significance. There is also some weak evidence from one small trial that among enzyme-enriched mouth care products, the more viscous one (BioXtra) can lessen xerostomia symptoms more than less viscous products (Oral Balance); however relevant effect size seems small (11.2mm difference on 0-100mmVAS) and clinical significance is unknown. With respect to the other 3 trials on salivary substitutes, there remains very weak evidence regarding the beneficial effects of an intra-oral device releasing Oral Balance gel, xanthan gum-based salivary substitutes, aloe vera gel, rape oil, or mucin spray upon xerostomia symptoms, salivary flow or QoL.

There present study has a number of limitations. The studies included in this review were conducted between 1993 and 2015. During this time the patient population has changed, and radiotherapy modalities have also changed. Although we could not attempt a separate analysis of patients who

underwent IMRT because of the lack of data in the literature, undoubtedly participants of the recent studies received lower radiation dosage with respect to those participating in studies two decades ago. The number of available treatment arms was also too low to estimate the impact of confounders. Also only summary data rather than patient level data were available. Finally, meta-analysis results on cevimeline should be read with caution as we detected moderate heterogeneity between the studies in the assessment of the mean difference in xerostomia. The two studies were conducted by the same research group so the heterogeneity is likely not due to variations in doses and application procedures, but rather to the baseline characteristics of the patients.

2.1.7 Conclusions

Pilocarpine and cevimeline should represent the first line of therapy in HNC survivors with radiotherapy-associated xerostomia and hyposalivation. There is very weak evidence that salivary substitutes can provide some, if any, benefit of small magnitude and unclear clinical significance. The use of other treatment modalities cannot be supported on the basis of current evidence.

2.1.8 Appendix

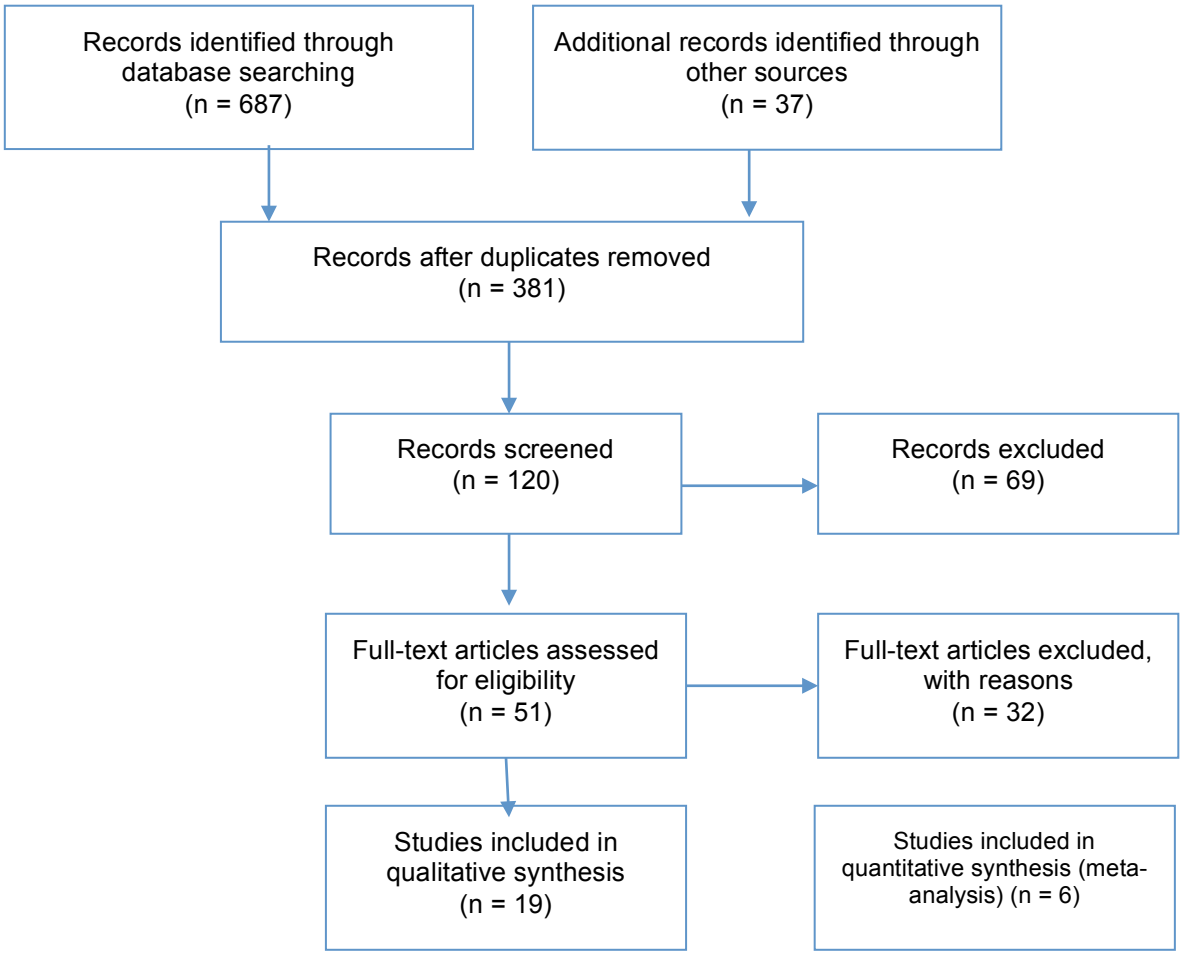


Figure 2-1 Flow Chart Systematic Review Strategy Search

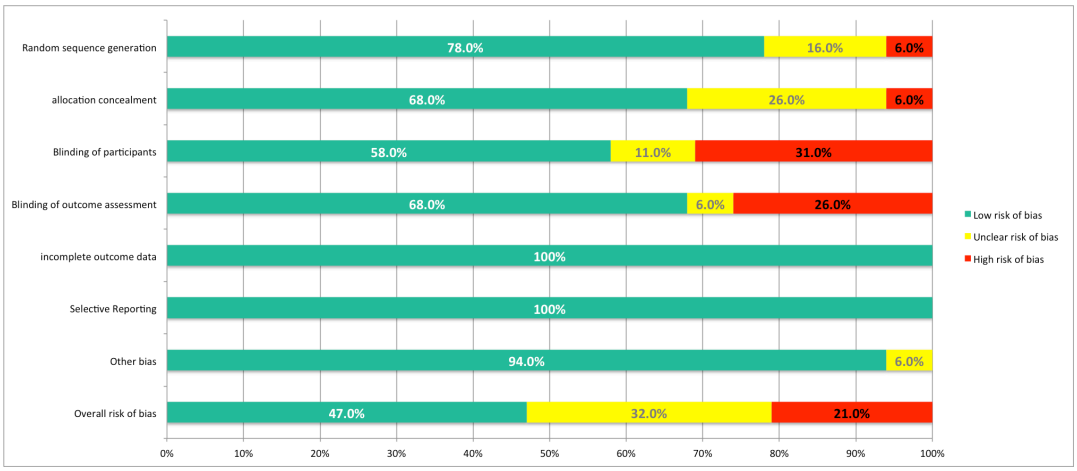


Figure 2-2 Risk of bias summary

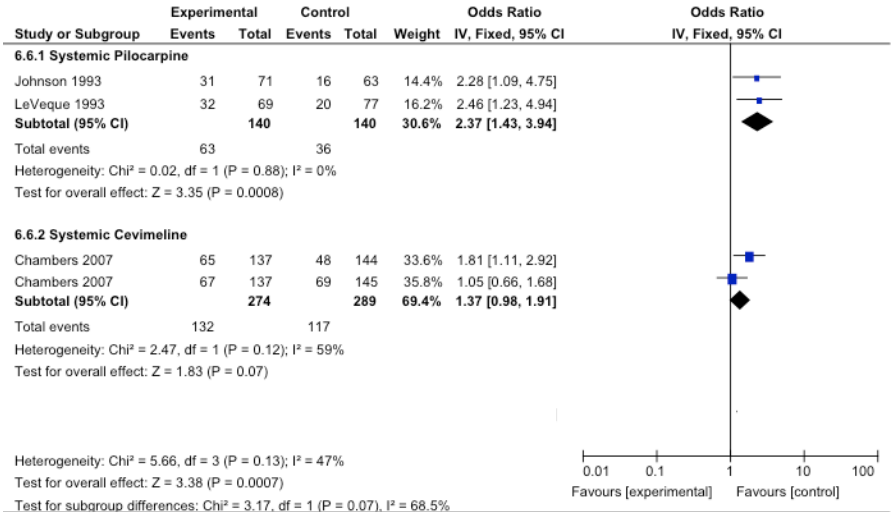


Figure 2-3 Forest Plot Primary Outcome: Xerostomia symptoms reduction from RCT (categorical data)

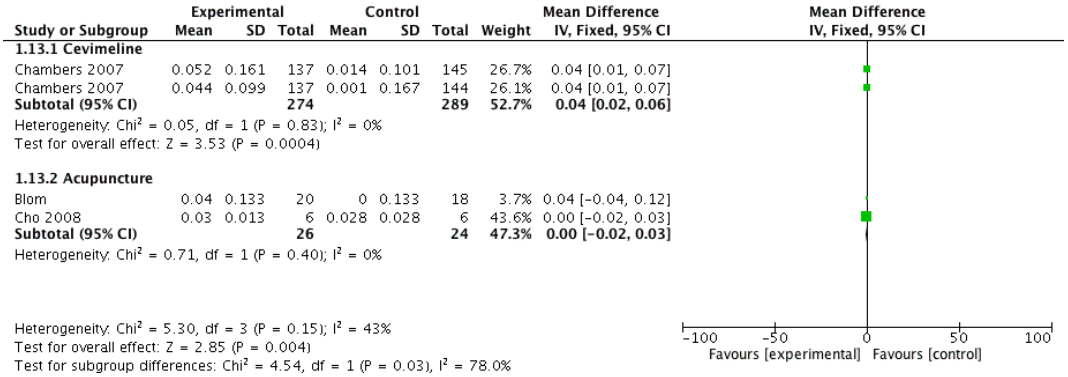


Figure 2-4 Forest Plot Secondary Outcome: Standardised mean difference in salivary flow rate

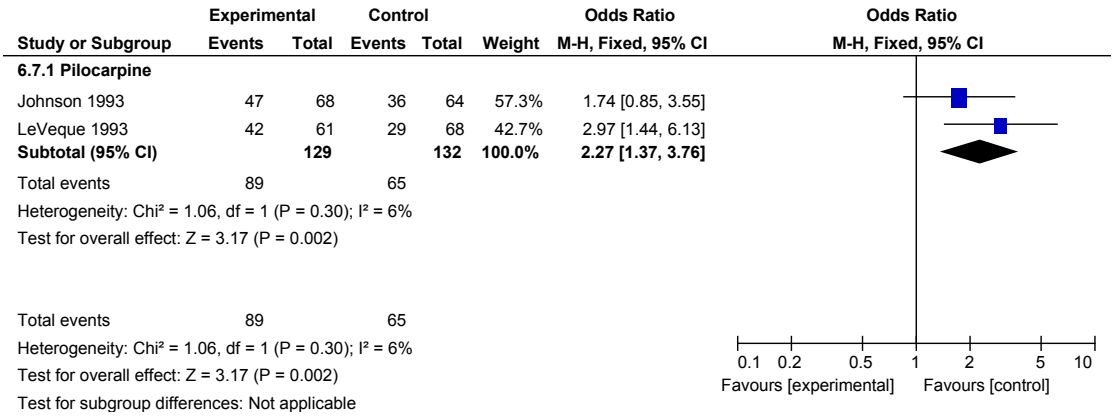


Figure 2-5 Forest Plot Secondary Outcome: Unstimulated salivary flow rate (categorical data)

2.2 Future Treatment for radiotherapy-associated xerostomia

2.2.1 Gene transfer

The term ‘gene transfer’ refers to the delivery of a gene to a predetermined target cell. Gene therapy can be applied to irradiated salivary glands to increase salivary secretion by enhancing the water permeability of duct cells thus creating an osmotic gradient. Transfer of genes into cells can be accomplished using viral and non-viral vectors (Samuni and Baum, 2011).

The delivery of DNA via non-viral vectors, which would reduce the safety risk, have scarcely been employed in the oral cavity, although current research is now directed toward a nanoparticle delivery (Arany et al., 2013).

Conversely, adenoviral-mediated aquaporin-1 cDNA (AdhAQP1) transfer has been studied and the results of the first open-label clinical trials were published in 2012. In this study the viral vector (AdhAQP1 vector) was administered to a single previously irradiated parotid gland in 11 subjects. The initial report showed that this approach is feasible and safe. A subset of patients (6 out of 11) showed enhanced parotid flow rates and reduction in xerostomia symptoms (Baum et al., 2012).

Currently, the same research group is planning to conduct a clinical trial using a less immunogenic vector derived from serotype 2 adeno-associated viral (AAV2), instead of the serotype 5 adenoviral vector used in the Phase I/Phase II study (Ad5) (Baum, 2014).

2.2.2 Stem cell therapy

A promising therapeutic approach for the management of radiotherapy-associated xerostomia is the stem cell therapy.

Theoretically, stem/progenitor cells could be harvested from salivary glands before radiotherapy in order to be implanted into the less damaged ductal complex, upon treatment completion (Vissink et al., 2015). Preclinical studies have shown that stem/progenitor cell transplantation can restore murine submandibular glands function (Lombaert et al., 2008).

2.2.3 Electrostimulation of salivary glands

The therapeutic potential of neurostimulation has been recognized in many areas of modern medicine, including the treatment of pain, deafness and cardiac arrhythmia.

The consideration that salivary gland function is controlled by the autonomic nervous system, has led to the application of neurostimulation in the management of salivary gland hypofunction and xerostomia.

Different strategies have been tested during the last three decades with variable outcomes. A comprehensive review of the literature on the topic is presented in the next section (2.3).

2.3 Electrostimulation of the salivary glands

2.3.1 Electrostimulation of salivary glands: rationale and mechanisms

Saliva secretion is controlled by dual innervation from the autonomic nervous system (Proctor and Carpenter, 2007). A reflex arch, comprised of three components, regulates saliva secretion: afferent nerves, central salivary nuclei, and efferent nerves.

Peripheral stimuli (associated by mastication, taste, smell and sight) are transmitted by the afferent nerve pathways from oral cavity and converge to the salivary nuclei in the medulla oblongata. The nuclei active an efferent

pathway, mediated by the parasympathetic and sympathetic components of the autonomic nervous system, which innervates the blood vessels and the acini of the glands and stimulates saliva production and excretion (Proctor and Carpenter, 2007). Parasympathetic and sympathetic nerves act separately but in coordination with each other. Parasympathetic system, inducing contraction of myoepithelial cells, evokes most of the salivary fluid secreted and it is responsible for the watery and electrolyte components of salivary secretion. It also causes vasodilatation, increasing glandular blood flow as part of the salivary reflex. The sympathetic stimulation causes vasoconstriction and produce a concentrated and viscous saliva rich in proteins (Garrett et al., 1991).

The therapeutic potential of electro-stimulating devices has been explored and recognised in many areas of modern medicine. Electro-stimulating devices are being used in the treatment of pain, deafness, bladder dysfunction, cardiac arrhythmia, muscular weakness, problems of the respiratory system and bone healing (Lafaurie et al., 2009).

Given the autonomic control of salivary secretion, a similar principle can be used in the management of salivary gland hypofunction. It is hypothesized that the application of electrical impulses on one of the components of the salivary reflex could improve salivary secretion and xerostomia symptoms.

Animal experiments have showed that the application of an electrical current through the oral mucosa on afferent receptors can enhance salivary production (Izumi and Karita, 1995, Kyriacou et al., 1988).

As the effects of electrostimulation were reported to sustain for as long as 6 months beyond cessation of therapy, it has been suggested that the

stimulation of the autonomic nervous system may cause the release of specific neuropeptides with trophic effects to salivary gland parenchyma, leading to regeneration of functional tissue. Animal studies have indeed demonstrated a mitogenic response in rat parotid and submandibular glands following electrical stimulation of their parasympathetic nerves (Mansson et al., 1990, Ekström and Reinhold, 2001, Calafat et al., 2009, Konttinen et al., 2011).

2.3.2 First generation of salivary electrostimulating device

The first generation of salivary electrostimulating device is represented by the Salitron (Biosonics, Fort Washington, Pa, USA) (Figure 8-1). The electrostimulating system consisted of an electronic control module and a hand-held stimulus probe with two stainless steel electrodes. The electrodes were positioned between the tongue and the palate of the mouth for a few minutes in order to stimulate gustatory (IV cranial nerve), and tactile receptors (V cranial nerve), afferent pathways of the salivary reflex. In 1986 Weiss et al tested the first generation device in a group of patients with xerostomia due to different causes, including radiotherapy to the head and neck (Weiss Jr et al., 1986). This open-label uncontrolled study showed an improvement in oral wetness, measured by visual inspection, in 50% of patients. Looking in a more detail at the subgroup of individuals with radiation-associated xerostomia, the improvement was sustained for 11 out of 13, with all the patients reporting a subjective reduction in xerostomia symptoms as response to the stimulation.

A more robust study methodology and reliable outcome measures were employed by Steller and collaborators, who designed a placebo-controlled

clinical trial and assessed device efficacy through sialometry (Steller et al., 1988). Five out of thirteen Sjögren's syndrome patients showed a reduction in xerostomia symptoms, which however was objectively demonstrated only in three.

The same device was studied by Talal et al in a multicentre, double blind study which included 77 Sjögren's patients (Talal et al., 1992). The electrostimulation group showed a difference of 116% greater than the placebo group between the pre and post-stimulation salivary production.

Given the promising results of these initial studies, the device was approved by the US Food and Drug Administration in 1998. However cost and bulky dimension have limited its wider diffusion within the medical community.

2.3.3 Second generation of salivary electrostimulating device

To overcome the limitations of the first-generation device, a EU-funded Consortium developed and tested a novel second-generation electrostimulating device, Saliwell GenNarino (Figure 8-2, 8-3,8-4). The novel device is a removable intraoral thermoplastic polyurethane-made appliance similar to mouth-guards (splints) used by individuals with temporomandibular disorder. It is custom made on the individual patient by using their teeth pattern moulds and its size is much smaller than the Salitron.

The electrical circuit and the battery have been miniaturized and are now embedded within the device, to avoid saliva contamination. Two electrodes protrude through the appliance to deliver the electric impulses to the trigeminal and lingual nerves via the oral mucosa. The device is suggested to stimulate the afferent and efferent neural pathways controlling submandibular, sublingual and parotid gland, as well as minor salivary

glands. An external remote control regulates device function, by means of infrared light transmission at a wavelength of 940nm-950nm (Fedele et al., 2010).

The efficacy of the novel device was initially tested in a small feasibility study on 23 patients with xerostomia associated with Sjogren's syndrome, medications, and of idiopathic nature. This was a double-blind crossover, sham-controlled multicentre trial aimed at investigating safety and short-term effectiveness of the device (Strietzel et al., 2007). The investigated device was equipped with a wetness sensor, embedded within the appliance, to record real-time changes of wetness during stimulation. The experiment consisted of one active stimulation test and one sham test for 10 minutes each, in a random order, followed by 10-minutes measurement period. After the performance of 158 experiments, a significant reduction of the oral dryness for the active mode was objectively registered by the wetness sensor, as well as subjectively by patients' judgement. The device was well tolerated by all participants and did not give rise to adverse side effects. The second-generation device was granted CE mark on the basis of the results of this initial pilot study.

A larger multicentre longer-term clinical trial was performed in 2011. Strietzel and al designed a randomised, multicentre crossover sham-controlled double-blind trial with primary endpoint being defined as a subjective improvement in the severity of xerostomia, assessed by visual analogue scale (Strietzel et al., 2011). Unstimulated and stimulated salivary flow rates were the second outcome measures of this study, assessed though sialometry. The randomised controlled trial had a 2-month duration and was

followed by a subsequent uncontrolled phase aimed at investigating long-term effectiveness. In the initial randomised double-blind phase, the electrostimulating device was used for 10 minutes at a time, each for 1 month, in either sham mode or active mode. The analyses on 114 patients completing the randomised controlled phase showed a better performance in terms of dryness severity (subjective symptoms) for the active intervention than for the sham experiments. No statistical difference, however, was found with respect to unstimulated and stimulated flow rates and oral discomfort. The subsequent second open-label phase consisted of a 3-month investigation of active devices in a subset of patients (n=79). Approximately 70% of treated individuals reported improvement in dryness severity and 63% of them showed increased unstimulated salivary flow rates at the end of 3-month treatment period. No changes were detected regarding stimulated salivary flow and quality of life scores.

Alajbeg at el reported the outcomes of extended long-term follow up (after 11 month of device usage) confirming the previous positive outcomes (Alajbeg et al., 2012). In 2014 a case series has been published reporting on six patients with chronic-versus-host disease (cGVHD) treated for 4 weeks with an intra-oral electrostimulator device and then randomly crossovered to a sham-device or vice versa, with patients and clinicians blinded to the intervention. The device proved to be safe in this cohort of patient, although two patients did develop mild mucosal lesions in areas in contact with the study device. Unstimulated and stimulated salivary flow and subjective sensation of xerostomia improved in 4 out of the 5 patients included after the two interventions. The limited number of participants did not allow any inferential statistics (Zadik et al., 2014).

2.3.4 Third generation electrostimulating device

In addition to the intra-oral removable appliance (second generation device), the EC-founded Consortium also developed a miniaturised device having the dimension and shape of a molar tooth and designated to be applied onto an osteointegrated implant (the Saliwell Crown) (Figure 8-5).

It was suggested that the use of a fixed device mounted on an osteointegrated dental implant would overcome the need of repeated application associated with the removable second-generation device, allowing constant stimulation of salivary glands without interfering with regular oral function. The implantable device is also equipped with an embedded wetness sensor in order to release electrical stimuli according the degree of oral dryness/wetness. A remote control would allow patients' control of electronic stimuli. The device is designed to be implanted in the lower third molar region, in close proximity to the lingual nerve and in order to avoid negative cosmetic impact.

In a clinical case report Ami et al investigated the effectiveness of the implanted device in a 81-years old female patient with xerostomia not responding to topical treatment and unable to take pilocarpine because of the presence of contraindications to pharmacological cholinergic stimulation (Ami and Wolff, 2010). The authors reported a notable objective improvement of salivary flow, speech, swallowing and subjective improvement of oral dryness symptoms.

2.3.5 Extra-oral electrostimulating device

The ability of extra-oral transcutaneous electric nerve stimulation to stimulate salivary glands, increase salivary secretion and lessen xerostomia symptoms

has been investigated using transcutaneous electrical nerve stimulation (high frequency, low intensity stimulation) and acupuncture-like transcutaneous electrical nerve stimulation (low frequency, high intensity stimulation).

The mechanism of action of extra-oral neurostimulation is not fully understood. It has been postulated that transcutaneous electric nerve stimulation (TENS) could directly stimulate the auriculotemporal nerve, which supplies the parotid gland, whereas it remains unclear whether there is also an indirect action (via afferent pathways) onto the salivary reflex arch. Hargitai in 2005 conducted a pilot study to assess the effectiveness of a TENS unit to stimulate parotid salivary flow in 22 healthy participants (Hargitai et al., 2005). The TENS unit used in this study was an AdvanTew 2000 (Amrex-Zetron, Carson, Calif), with the electrode pads placed on the skin overlying the parotid glands. Fifteen of the 22 adults showed an increase in the parotid flow rate, assessed through a 5- minutes collection of parotid saliva with Carlson-Crittended cup before and after stimulation. Three patients experienced fasciculation and anaesthesia of the area of pad application, which ceased with the cessation of TENS application.

A recent prospective phase II study evaluated the effectiveness of TENS technique in 30 patients with radiation-induced xerostomia, showing that in all patients but one an increased salivary flow after stimulation (Vijayan et al., 2014).

Acupuncture-like TENS (ALTENS) has been tested in a phase 2 study recently conducted by Wong in patients with radiation-associated xerostomia (Wong et al., 2012) and the results of this study have been described previously in the systematic review.

3 Knowledge gap

The use of intra-oral second-generation device may represent an ideal therapeutic strategy for individuals with RT-associated xerostomia. The device sits comfortably in the patient's mouth and can be easily applied and removed; it stimulates natural salivation with no notable adverse side effects. The potential trophic effects on glandular parenchyma have been described (Schneyer et al., 1993).

The evidence supporting its efficacy is however limited. Available studies have included very few participants with RT-associated xerostomia. More importantly, the design and overall quality of published clinical trials is debatable.

As a consequence it remains unclear whether the second-generation intraoral electrostimulating device is indeed effective in providing (i) long-term reduction in xerostomia symptoms and (ii) increase of salivary flow. It is also unknown whether it could lead to a significant improvement in quality of life.

Finally, the magnitude of any potential benefit is also unclear.

This PhD thesis describes the aims, design and results of a multicentre clinical investigation on the second-generation intraoral electrostimulating device in individuals with RT-associated xerostomia, the LEONIDAS-2 clinical trial.

4 The LEONIDAS-2 Clinical Trial

4.1 Aims

LEONIDAS-2 (**L**ong-term **E**valuation of the effectiveness **O**f a **N**ovel **I**ntra-oral electro-stimulator for the treatment of ra**D**iotherapy-**AS**sociated xerostomia) is a study funded by the National Institute of Health and Research (NIHR) (ISRCTN: 24437812) aimed at testing the hypothesis that long-term application of the second generation electrostimulating device in patients with radiation-associated xerostomia will (i) reduce xerostomia symptoms, (ii) improve salivary function, and (iii) improve quality of life.

4.2 Methods

4.2.1 Trial design

LEONIDAS-2 is a double blind multicentre controlled randomised trial of 12-months therapy, during which patients have been randomly assigned to treatment with a fully functioning electrostimulating device or a sham device (releasing mechanical but not electrical stimulation).

4.2.2 Participants

Patients with radiotherapy-induced xerostomia attending the UCLH Oral Medicine Unit, the H&N Cancer Centre of UCL/UCLH, the Maxillofacial/H&N Surgery Unit at Bradford Royal Infirmary, the Head and Neck Unit of the Royal Marsden have been considered for recruitment.

The study has taken place at the Eastman Clinical Investigation Centre of Eastman Dental Institute and Hospital (University College London and University College London Hospitals Trust) and Bradford Royal Infirmary. Participants have been recruited on the basis of inclusion and exclusion criteria presented in Table 4-1.

Table 4-1 LEONIDAS-2 Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
(i) Be at least 18 years old	(i) Severe systemic disease (on the basis of the classification of the American Society of Anaesthesiology: ASA III and ASA IV) (Table 4-3)
(ii) Have received more than 40 Gy of external beam RT for cancer in the H&N region at least 4 months before entry into the study	(ii) Known allergy to materials similar to those used in the investigational product
(iii) Have grade 1 or 2 of RTOG/EORTC Late Radiation Morbidity Scoring Schema (Table 4-2)	(iii) Wearing of other active implants such as cardiac pacemaker or defibrillator, or hearing aids
(iv) Have a degree of minimum degree of dryness of 50mm (≥50mm) on a 100mm VAS scale (0=no dryness; 100 =maximum dryness).	(iv) An unstimulated whole salivary flow of 0ml/5min (complete absence of unstimulated salivary flow as measured via sialometry for 5 minutes).
(v) Have demonstrable residual salivary gland function (increase in salivary flow on appropriate stimulation (e.g. chewing paraffin wax)	(v) Use of pilocarpine as systemic therapy
(vi) Have at least one parotid gland	(vi) Grade 3 RTOG/EORTC or no resting saliva (sialometry = 0mL/1.5 min)

Table 4-2 RTOG/EORTC Late Radiation Morbidity Scoring Schema (salivary glands)

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
None	Slight Dryness Of Mouth Good Response On Stimulation	Moderate Dryness Of Mouth Poor Response On Stimulation	Complete Dryness Of Mouth No Response On Stimulation	Fibrosis	Death

Table 4-3 American Society of Anaesthesiology (ASA) Physical Status

Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
A normal healthy patient	A patient with mild systemic disease	A patient with severe systemic disease	A patient with severe systemic disease that is a constant threat to life	A moribund patient who is not expected to survive without the operation	A declared brain-dead patient whose organs are being removed for donor purposes.

4.2.3 Primary and secondary outcomes

The primary outcome of the study was defined as the proportion of participants reporting a 30% reduction of xerostomia symptoms after 12 months of treatment, as evaluated through a 100mm VAS (100mm=maximum dryness) based on changes between measurements

recorded at admission (baseline, month 0) and the end of the trial (after treatment, end of month 12).

Secondary outcomes included:

- Xerostomia symptom VAS scores [end of month 12 and repeated measurements up to 12 months]
- Salivary flow rate [end of month 12 and repeated measurements up to 12 months]
- EORTC QLQ-H&N35 (Head and Neck cancer QoL) questionnaire [end of month 12 and repeated measurements up to 12 months]
- Oral health-related quality-of-life measure (OH-QoL16) questionnaire [end of month 12 and repeated measurements up to 12 months]
- Short Form-36 (SF-36) questionnaires (QoL) [end of month 12 and repeated measurements up to 12 months]
- Frequency of usage of the device (from a daily diary of device usage)

Patient data were collected at screening and baseline (visit 2, delivery of the individualised device) and at follow-up appointments at the end of months 1, 2, 4, 6, 8 and 12 after the start of treatment.

A clinical trial of 12 months has been designed in order to provide new data on the effectiveness and safety of this treatment over the long-term, as previous clinical trials have investigated the effectiveness and safety of a 9-month intervention.

4.2.4 Outcome measures

Patient-centred outcome measure

The 100-mm long Visual Analogue Scale, previously described, has been chosen as the primary outcome measure in LEONIDAS-2 because it is a patient-centred outcome and the most frequently used self-rating instruments for the measurement of xerostomia.

There are no studies determining the levels of change on standard xerostomia scales that represent clinically important differences to patients.

The cut-off of 30% has been chosen in this study because of the observed minimum clinically significant improvement in pain studies (33%) (Farrar et al., 2000), and in order to promote homogeneity across studies on xerostomia who used the same outcome measure (Brown et al., 2014, Mariette et al., 2015). A critical analysis of the literature performed during the study to estimate the magnitude of change of the visual analogue scale in head and neck settings, has showed that the magnitude of VAS change (effect size) following the intervention could be more informative for decision-makers than the dichotomous presentation. We have therefore added the analysis of xerostomia symptom though continuous VAS scores as secondary outcome measure.

Clinician-rated outcome measure

Sialometry has been discussed in the section: “Diagnosis and Assessment of radiotherapy-associated xerostomia”.

Quality of Life assessment

EORTC QLQ-H&N35

The European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Study Group has developed a measurement strategy for the assessment of quality of life (QOL) in clinical trials (Bjordal et al., 1994a, Bjordal et al., 2000). The QLQ-H&N35 includes 35 items, 7 multi-item scales, measuring pain in the mouth, problems with swallowing, senses, speech, social eating and social contact, and 11 single-item scales, assessing

problems with teeth, mouth opening, xerostomia, sticky saliva, coughing, feeling ill, as well as use of analgesics, nutritional supplements, feeding tube, weight gain and weight loss. The time frame of the module is ‘‘during the past week,’’ and items are scored either on four-point Likert-type categorical scales (‘‘not at all,’’ ‘‘a little,’’ ‘‘quite a bit,’’ ‘‘very much’’) or have a ‘‘no/yes’’ response format. The scores are transformed into 0-to-100 scales, with a high score implying a high level of symptoms or problems. The module has been translated into 60 languages and is in use worldwide as one of the standard instruments for measuring quality of life in head and neck cancer patients. The reliability coefficients for most of the QLQ-H&N35 scales ranged from 0.61 to 0.93, indicating satisfactory internal consistency (Singer et al., 2013).

The EORTC QLQ- H&N35 module has been chosen for this study because it is the most common tool to gather QoL data used in RCTs, and it proved to be well accepted by patients, with completeness of the questionnaire varying from 66 % to 99 % (Singer et al., 2013).

OH-QoL16

The impact of oral health on participant’s quality of life has been assessed using the UK Oral Health-related quality-of-life measure (OHQoL-UK), which consists of 16 key questions on key areas of oral health related quality of life. Participants were asked to rate the impact of gums, mouth, and/or denture on their overall quality of life. The overall score scores ranging from 16 (all bad effects of extreme impact) to 144 (all good effects of extreme impact). This instrument was chosen for the good psychometric properties showed in the validation study (McGrath and Bedi, 2001).

Short Form-36 (SF-36) questionnaires

The SF-36 questionnaire has been selected because it is a valid and reliable instrument to assess general health and the most widely used QoL instruments in clinical trials to measure physical and mental health. Its measures eight domains: physical and social functioning, role limitations due to physical health or emotional problems, fatigue, emotional well-being, pain and general health. A single item is also included to assess the perceived change in health over time (Ware, 2000). Each scale is directly transformed into a 0-100 scale, with an overall scores ranging from 0 (worst possible level of functioning) to 100 (best possible level of functioning).

4.2.5 Intervention

The device tested in this clinical trial is the second-generation of intra-oral removable devices named Saliwell GenNarino, consisting of a mouthpiece made to fit the mandibular dental arch and an infrared remote control to activate or deactivate the electrical stimulation. The device contains an electronic circuit with a microprocessor and a receiver of remote control signals, a pair of stimulating electrodes, and a 30-mA/h battery. The electrodes contact the oral mucosa in the mandibular third molar area, close to the lingual nerve; therefore no conductive gel is needed. A green light on the intra-oral device (either active or sham) blinks upon activation of the remote control to show that the device received the signal from the remote.

The electrostimulating devices have been manufactured for each participant using impressions taken from the dental arches (Manufacturer: Saliwell Ltd, Israel). Participants have been randomly assigned to:

- Group A (cases): patients who receive a fully functioning electrostimulating device.

- Group B (controls): patients who receive a device that does not release electric stimuli (but provides mechanical/tactile stimulation).

4.2.6 Experimental Procedure

The general structure of experimental procedures consists of:

A: Screening/Enrolment

B: Dental Impression

C: Delivery of individualised device

D: Follow-up appointments

(A) SCREENING/ENROLMENT

Potential participants identified during routine clinical sessions have been invited to attend an initial screening appointment. A careful assessment of the general health status, comprising of medical history and details of previous head and neck cancer treatment has been performed.

A dedicated anonymized Case Report Form has been used to gather clinical data. The forms have been stored at the UCL Eastman Dental Institute (EDI), London, and Bradford Royal Infirmary in locked facilities.

Data were collected for all screened patients to assess eligibility before offering randomisation. Information collected included patient date of birth, gender, ethnic group, pulse, blood pressure, smoking and drinking habits, site and stage of primary tumour, details of treatment for head and neck

cancer. The medical history included xerostomic medical condition and medication.

As the device is designed to stimulate the salivary glands, only patients demonstrating residual salivary gland function were included. For this purpose, unstimulated whole saliva (UWS) and paraffin chewing-stimulated whole saliva (SWS) were collected during the screening visit. All subjects were instructed to refrain from smoking, eating, drinking or tooth brushing at least 90 minutes prior to saliva collection. UWS was collected for 5 min using an established spitting technique. The collection time (5 minutes) was chosen because it is the commonly used interval, being reliable, practical for the clinician, and more comfortable for the patient (Villa et al., 2014). During the stimulated whole saliva collection, the subjects chewed a piece of tasteless parafilm (5 × 5cm, 0.30g; Parafilm, Fisher Scientific, UK) at their natural pace. Saliva volumes were determined gravimetrically (assuming 1g = 1mL), using pre-weighted tubes (Sterilin, UK, catalogue n.185CM) and a precision balance (Scout Pro SPU123, Ohaus, NJ), with saliva flow rates expressed in millilitres per minute (mL/min).

All assessments have been performed preferentially at a fixed time of the day to minimise fluctuations related to the circadian rhythm of salivary secretion.

The grade of xerostomia has also been assessed using the dedicated *RTOG/EORTC Late Radiation Morbidity Scoring Schema* (Table 4-2) and xerostomia VAS score.

If entry criteria were satisfied, patient were provided with the relevant Patient Information Sheet (Appendix 8.2) and given all the time they need to consider participation, ask further questions and provide written consent.

(B) IMPRESSION and MANUFACTURING

A full dental impression of the lower arch has been taken by the clinical investigators. Impression tray of the lower arch was carefully selected considering the potential limitation of mouth opening due to the fibrosis induced by the radiotherapy and the need to extend the impression to the distal and lingual aspect of the third molar area (at least 1 cm away in each direction). To ensure stability of the impression, polyvinylsiloxane materials (Aquasil Ultra Medium-Heavy Body, Dentsply) have been used with a one-stage impression technique. In case of lower removable prosthesis the impression was taken after removal of the prosthesis, in order to ensure intimate and stable contact of the electrodes with the tissue. The impression was rinsed thoroughly under running tap water before being soaked for 10 minutes in a disinfecting solution (Eurosept Max Impressions Powder, Henry Schein). The impression was then rinsed again, air-dried and labelled with the randomisation code before being sent to the manufacturer.

(C) DELIVERY OF INDIVIDUALISED DEVICE

During the delivery of individualised device the participants have been instructed and trained by the clinical investigators regarding the modality of use of the device during the study period. Saliwell GenNarino was disinfected with sterile gauze and 70% alcohol and the device gently inserted in the oral cavity, commencing with the side containing the electrodes and then the other side. If the participant reported discomfort, fine adjustments were made to release stressful spots inside the device by removing a thin acrylic layer with a handpiece. In case the patient reported a pricking sensation for the point of contact with the electrodes, these were slightly shortened using a

high-speed turbine. Careful attention was paid to not excessively trim the electrodes, thus annulling the stimulating effect. Saliwell GenNarino was activated by pressing the green button of the personal remote control, pointing its front to the Saliwell GenNarino detector at a distance of 2-10 cm. The blink of the detector light was sought in order to confirm that the device was working properly.

Patients were asked to use the device with a frequency of 5 minutes/hour, as many times as they wanted during the day.

Following appropriate training of the participant on the safe use of the device, written manufacturers' instructions were provided, and contact details of the investigators were given to the participants.

Participants were asked to complete a diary of the frequency of application of the device per day (Appendix 8.3).

At baseline the following data were recorded: xerostomia symptoms (VAS scale), sialometry, EORTC QLQ-H&N35, OH-QoL16, SF-36 questionnaires.

(D) FOLLOW-UP

During follow-up appointment (end of months 1, 2, 4, 6, 8 and 12 after the delivery of the individualised device to the patient), the presence of oral mucosal abnormality and salivary gland swellings were recorded, together with xerostomia symptoms, sialometry, EORTC QLQ-H&N35, OH-QoL16 and SF-36 questionnaires. Safety-related secondary outcome measures and changes in health condition were assessed.

4.2.7 Samples size

A sample size of 84 subjects has been calculated with regard to the primary outcome. Assuming that 20% of the patients of the control group and 60% of patients on the active group reported the successful outcome (30% reduction in symptoms on the VAS), the sample size required to detect such a difference in reduction of xerostomia symptoms with 90% power using a cut-off for statistical significance of $p<0.05$ was 70. Considering a potential drop out of 20%, the total sample size reached 84 participants (42 patients in the study group and 42 in the control group). Drop out rate was primarily estimated on the basis of expected mortality/recurrence of head and neck cancer in the first 18 months after treatment and study duration.

4.2.8 Randomisation

Sequence generation

A blocked random list, allocating the patient identification numbers to the specific type of stimulation, has been prepared by an independent study coordinator (central randomisation). The method of sequence generation was a random-number generator on a computer. The randomisation code has been disclosed only at the end of the trial.

Allocation concealment

Allocation concealment, aimed at keeping clinicians and participants unaware of upcoming assignments, was guaranteed as the computer-generated list was prepared and maintained by the independent study coordinator based at a different study centre. The independent study coordinator had no contact with the patients and has undertaken the randomisation, allocated the study devices, and held the trial codes.

Implementation

The implementation process has been performed by clinical researchers at clinical centres, who assigned to enrolled participants the next available study number and had no contact with the independent study coordinator.

4.2.9 Blinding

The study was a double blind controlled randomised trial. Neither clinical investigators nor participants knew which devices were active and which were not. The double-blind design was feasible as the electronic stimulus was completely asymptomatic, thus the participant could not realise whether or not they were using an active or sham device.

4.2.10 Statistical analysis

Statistical support and supervision for this study has been provided by a dedicated trial statistician. All statistical tests and confidence intervals were 2 sided and significance has been considered at the 5% level and confidence intervals at the 95% level. Statistical analyses have been performed using STATA software (version 14).

The primary outcome of proportions of patients reporting ≥ 30% reduction (from baseline) in xerostomia symptoms at 12 months (using a 100mm VAS with 0=no dryness and 100 = maximum dryness) has been compared between the randomised groups using a Fisher's exact test. Logistic regression models has been fitted to adjust odds ratios for centre, age, gender and other potential prognostic factors including type of radiotherapy (IMRT vs. conventional), total dosage of radiotherapy to primary tumour, fields of radiotherapy, number of residual salivary glands, type of tumour and years since radiotherapy completion.

For secondary outcomes (continuous 12-month VAS scores, 12- months sialometry and quality of life questionnaires), data have been compared between randomised groups using appropriate regression models to adjust for baseline data, providing estimates of adjusted treatment effect with 95% confidence intervals. Models have been extended to incorporate the repeated measures at 1,2,4,6,8 months allowing estimation of the average treatment effect achieved over the 12 month follow up. Changes in the effect of treatment over time on the VAS scores, salivary flow and quality of life scores have been initially examined graphically using individual patient response profiles plotted against time. Secondary outcomes have been adjusted for the prognostic factors used for the primary analysis.

All analyses have been carried out on an intention-to-treat basis, including all the participants according to their original randomised group assignment.

The average number of device applications used by patients per day has been summarised graphically over time within the intervention and control groups.

Information about adverse events has been summarised descriptively.

5 Results

5.1 Recruitment results

One hundred and nineteen patients with radiotherapy-associated xerostomia were screened between the Eastman Dental Institute (UCL) and Bradford Royal Infirmary. The recruitment took place between January 2012 and November 2013. Twenty-six participants were not eligible and were not allocated to the intervention (6 in UCL and 20 in Bradford Royal Infirmary). Seven participants were eligible but declined to participate in the study (Figure 5-1).

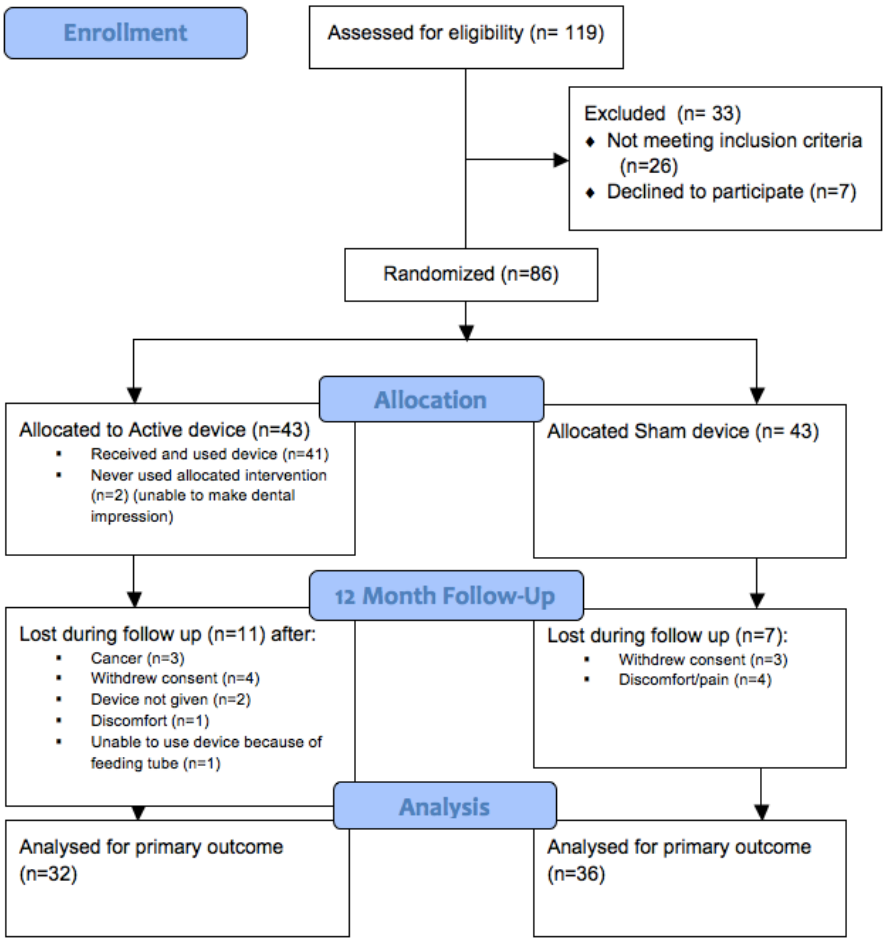


Figure 5-1 LEONIDAS-2 Consort Flow Diagram

We randomly allocated 86 participants from the two centres to treatment with either sham or active intra-oral electrostimulating device (43 patients to each group). Two participants, however, did not receive the allocated intervention because, due to the presence of trismus, it was not possible to take the dental impression needed for manufacturing the device.

Recruitment trend for each centre and combined is presented in figure 5-2, 5-3 and 5-4. Sixty-six participants were recruited at UCL and twenty (but two did not receive the allocated intervention) at Bradford Royal Infirmary.

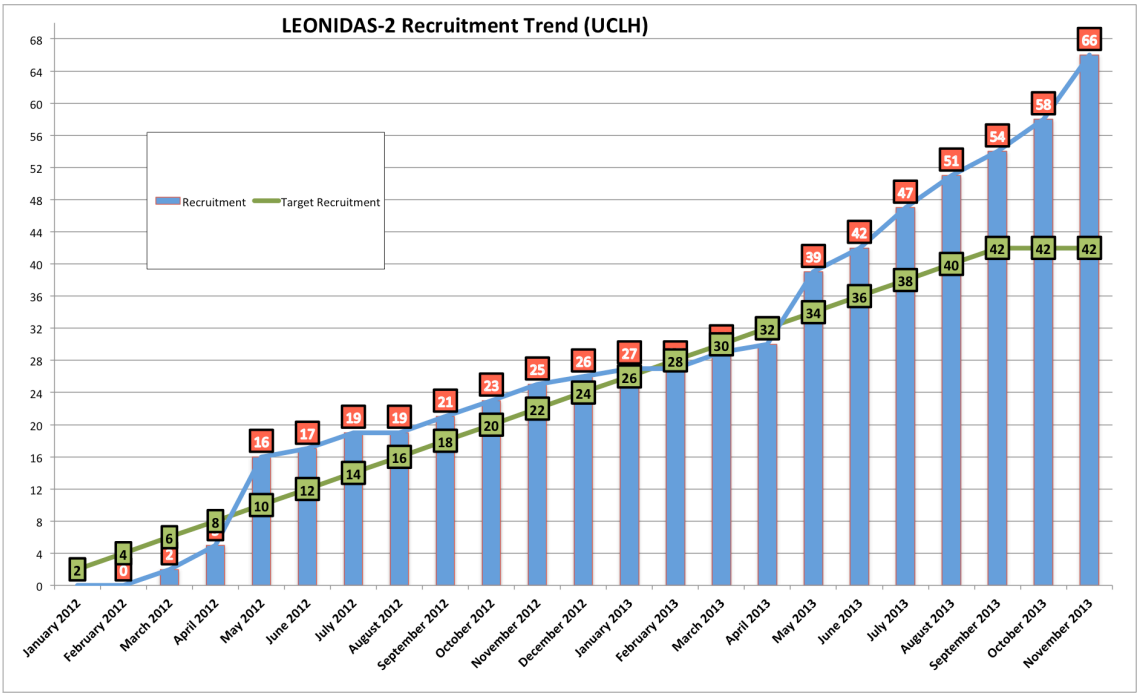


Figure 5-2 LEONIDAS-2 Recruitment trend (University College London)

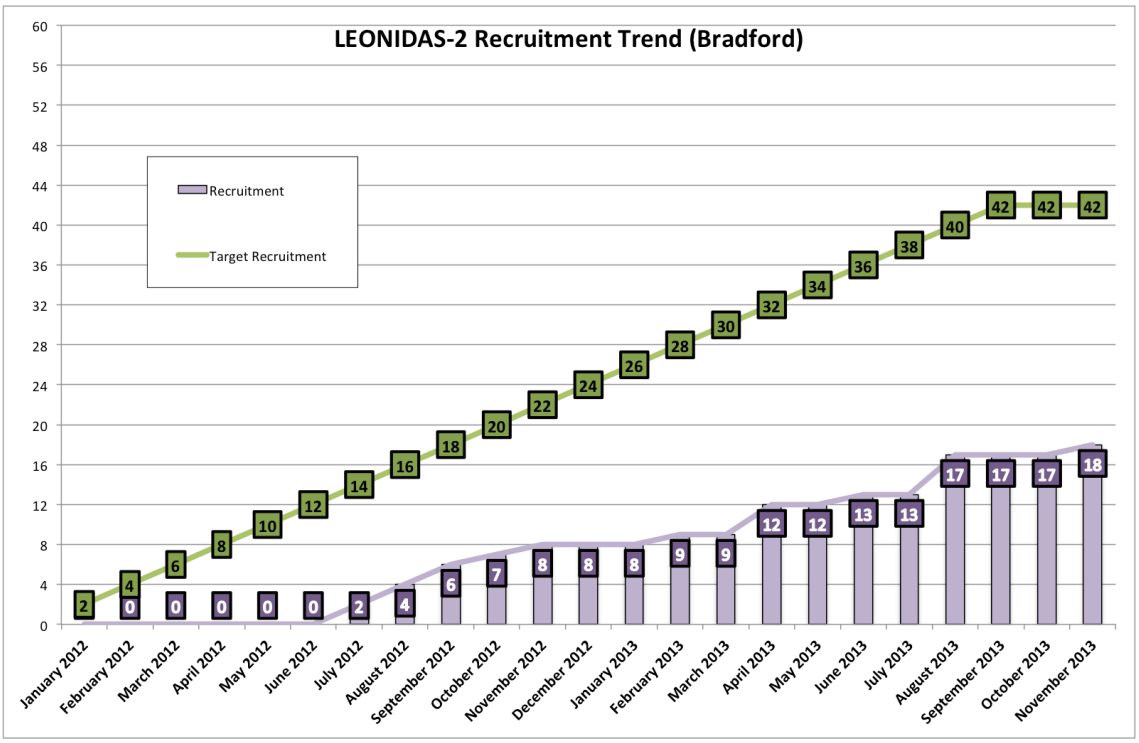


Figure 5-3 LEONIDAS-2 Recruitment Trend (Bradford Hospital)

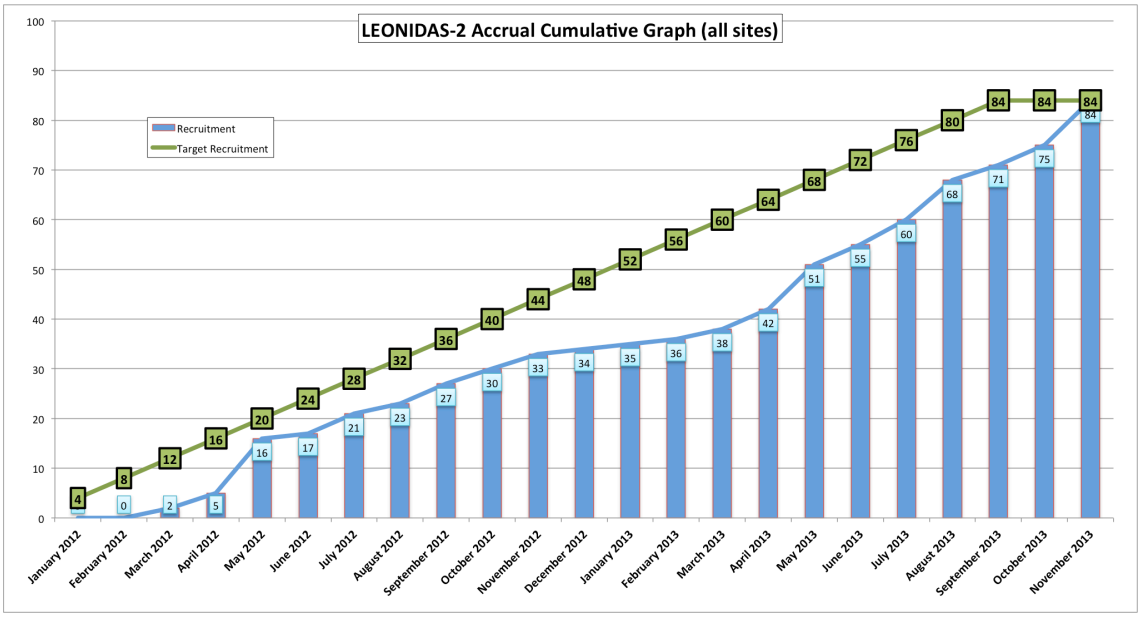


Figure 5-4 LEONIDAS-2 Recruitment Trend (all sites)

5.2 Baseline characteristics

Table 5-1 shows baseline characteristics and treatment details for each group. Baseline xerostomia severity (VAS) was slightly higher in the sham group then in the active group (7.44 for the sham group and 6.99 for the

active). Age, gender, radiotherapy regimen and dosage of radiotherapy to primary tumour were similar between groups.

Table 5-1 Baseline characteristics of patients randomised by treatment group.

		Sham pacemaker (n=43)	Active pacemaker (n=43)
Centre	UCLH	33 (77%)	33 (77%)
	Bradford	10 (23%)	10 (23%)
Age at baseline (years)	Mean (SD)	58.2 (9.3)	58.4 (10.8)
Gender	Male	31 (72%)	35 (81%)
	Female	12 (28%)	8 (19%)
Smoker	Yes	4 (9%)	5 (12%)
	No	39 (91%)	38 (88%)
Ethnic group	Caucasian	40 (93%)	38 (88%)
	Other	3 (7%)	5 (12%)
Alcohol (units/week) **(0 for non drinkers)**	Median (IQ range)	1 (0 to 5)	2 (0 to 5)
Alcohol drinker	Yes	23 (53%)	24 (56%)
	No	20 (47%)	19 (44%)
Site of first cancer	Oral cancer	7 (17%)	4 (10%)
	Oropharyngeal	22 (53%)	24 (57%)
	Pharynx	3 (7%)	1 (2%)
	Hypopharynx	2 (5%)	1 (2%)
	Larynx	1 (2%)	0
	Salivary gland	1 (2%)	2 (5%)
	Other	6 (14%)	10 (24%)
Stage of Primary Tumour	**I**	4 (11%)	4 (11%)
	II	2 (5%)	3 (8%)
	III	10 (28%)	5 (13%)
	IV	20 (56%)	26 (68%)
Type of Radiotherapy	Conventional	19 (44%)	18 (42%)
	IMRT	24 (56%)	25 (58%)
Dosage of radiotherapy to primary tumour [Gross Tumour Volume - GTV] (Gray)	Mean (SD) Median (IQ range)	64.37 (2.81) 65 (65 to 65) n=30	64.00 (3.27) 65 (65 to 65) n=31
Number of residual parotid glands	1	0	0
	2	43 (100%)	43 (100%)
Number of residual submandibular glands	1	5 (12%)	5 (12%)
	2	35 (88%)	38 (88%)
Years since Radiotherapy completion	Mean (SD) Median (IQ range)	4.71 (2.93) 3.5 (3 to 6) n=38	5.14 (2.92) 5 (3 to 6) n=43
Xerostomic medical condition	Yes	10 (23%)	6 (14%)

	No	33 (77%)	37 (86%)
Xerostomic medication	Yes	14 (33%)	13 (30%)
	No	29 (67%)	30 (70%)
Chemotherapy	Yes	37 (86%)	31 (72%)
	No	6 (14%)	12 (28%)
Surgery to primary site	Yes	25 (58%)	24 (56%)
	No	18 (42%)	19 (44%)
Salivary flow rate (ml/min) (at screening)	Median (IQ range)	0.07 (0.04 to 0.15)	0.09 (0.04 to 0.14)
Dry mouth symptoms VAS (at screening)	Mean (SD)	7.44 (1.59)	6.99 (1.47)
EORTC QLQ-H&N35			
Swallowing	Mean (SD)	29.34 (23.94) n=41	29.56 (21.08) n=42
Senses	Mean (SD)	37.40 (27.08) n=41	37.30 (29.40) n=42
Speech	Mean (SD)	24.93 (23.14) n=41	23.02 (18.43) n=42
Social eating	Mean (SD)	38.62 (28.67) n=41	39.48 (26.93) n=42
Dry mouth	Not at all/ a little	4 (10%)	3 (7%)
	Quite a bit/ very much	37 (90%)	39 (93%)
Sticky saliva	Not at all/ a little	18 (44%)	20 (49%)
	Quite a bit/ very much	23 (56%)	21 (51%)
Coughing	Not at all/ a little	33 (80%)	30 (71%)
	Quite a bit/ very much	8 (20%) n=41	12 (29%)
OH-QoL16 overall score	Mean (SD)	44.09 (7.45) n=39	45.43 (10.35) n=42
SF36			
General health perception	Mean (SD)	56.55 (21.93) n=40	56.87 (19.73) n=42
Physical role functioning	Mean (SD)	46.79 (41.82) n=39	57.74 (40.76) n=42
Social role functioning	Mean (SD)	73.13 (23.78) n=40	74.11 (26.39) n=42
Mental health	Mean (SD)	72.53 (15.75) n=40	70.57 (20.22) n=42
Reported health transition	Better than a year ago	25 (63%)	27 (64%)

The mean age of participants was 58.2 for the sham group and 58.4 for the active group (Figure 5-5).

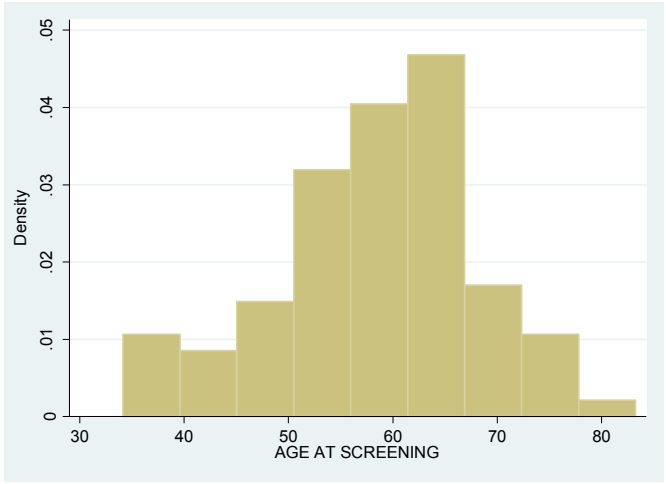


Figure 5-5 Histogram of baseline age

Fifty per cent of the participants declared to drink alcohol, with an average of 1 unit for week for the sham group and 2 units for the active group (Figure 5-6).

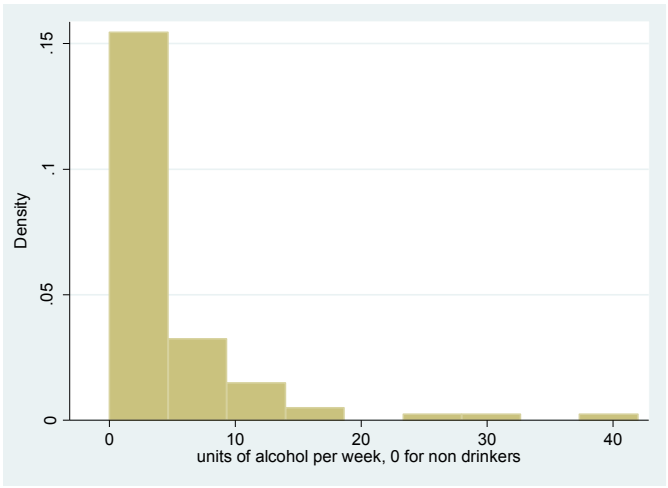


Figure 5-6 Histogram of baseline alcohol units per week

The majority of participants in both groups received IMRT (56% in the sham group vs. 58% in the active group), with a mean dose to the primary tumour of 64Gy (Figure 5-7).

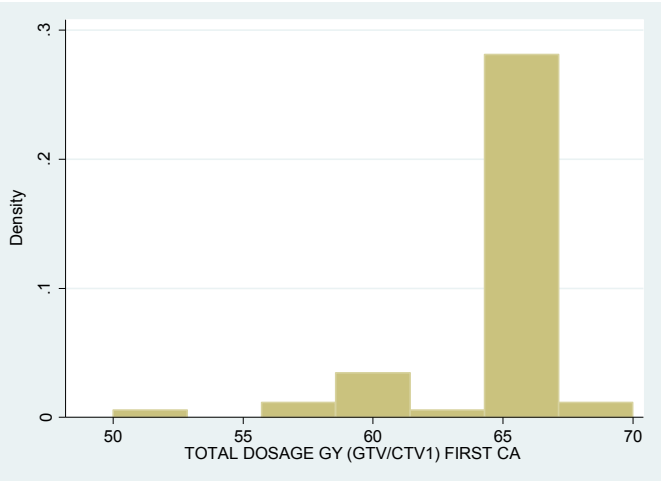


Figure 5-7 Histogram of dosage of radiotherapy to primary tumour

For inclusion criteria, patients having completed the radiotherapy at least 4 months before the screening visit were eligible, with no other time restraint. In this trial, participants had completed the radiotherapy on average 4.71 years for the sham and 5.14 for the active group before starting the clinical trial (Figure 5-8).

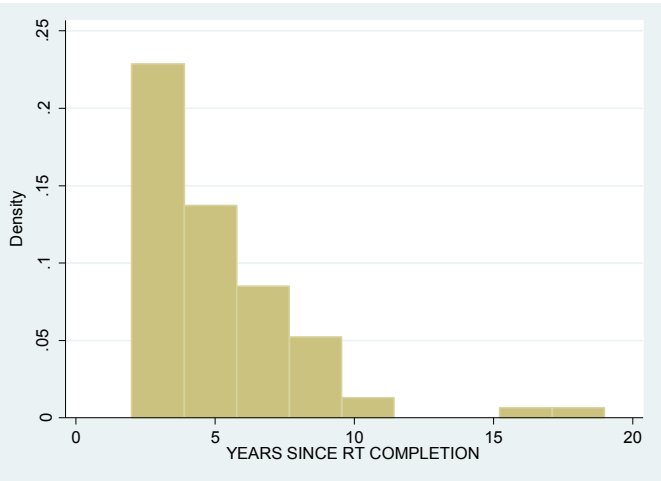


Figure 5-8 Histogram of years since radiotherapy completion

During the screening visit, eligible participants had to show to have demonstrable residual salivary function (increase in salivary flow on appropriate stimulation) and an unstimulated whole salivary flow higher than

of 0ml/5min (complete absence of unstimulated salivary flow as measured via sialometry for 5 minutes). The median unstimulated salivary flow was 0.07 ml/min for the sham device and 0.09 ml/min for the active (Figure 5-9).

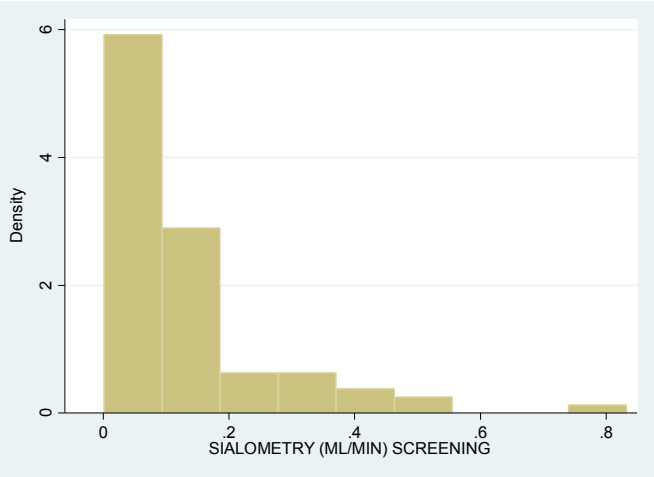


Figure 5-9 Histogram of baseline salivary flow rate (ml/min)

The Figure 5-10 shows that LEONIDAS-2 participants had a degree of dryness higher than the inclusion criteria (minimum degree of dryness of 50mm (≥50mm) on a 100mm VAS scale (0=no dryness; 100 =maximum dryness).

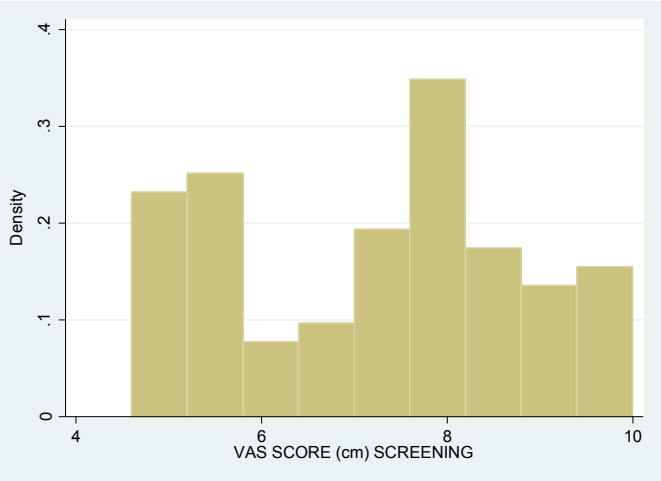


Figure 5-10 Histogram of baseline dry mouth symptoms (VAS)

For the EORTC QLQ-H&N35 questionnaire four multi-item sub-scale score (swallowing, senses, speech, social eating) and three single item sub-scale scores (dry mouth, sticky saliva, coughing) were analysed. The histograms of the baseline mean score for the multi-item sub-scales are reported in Figure 5-11.

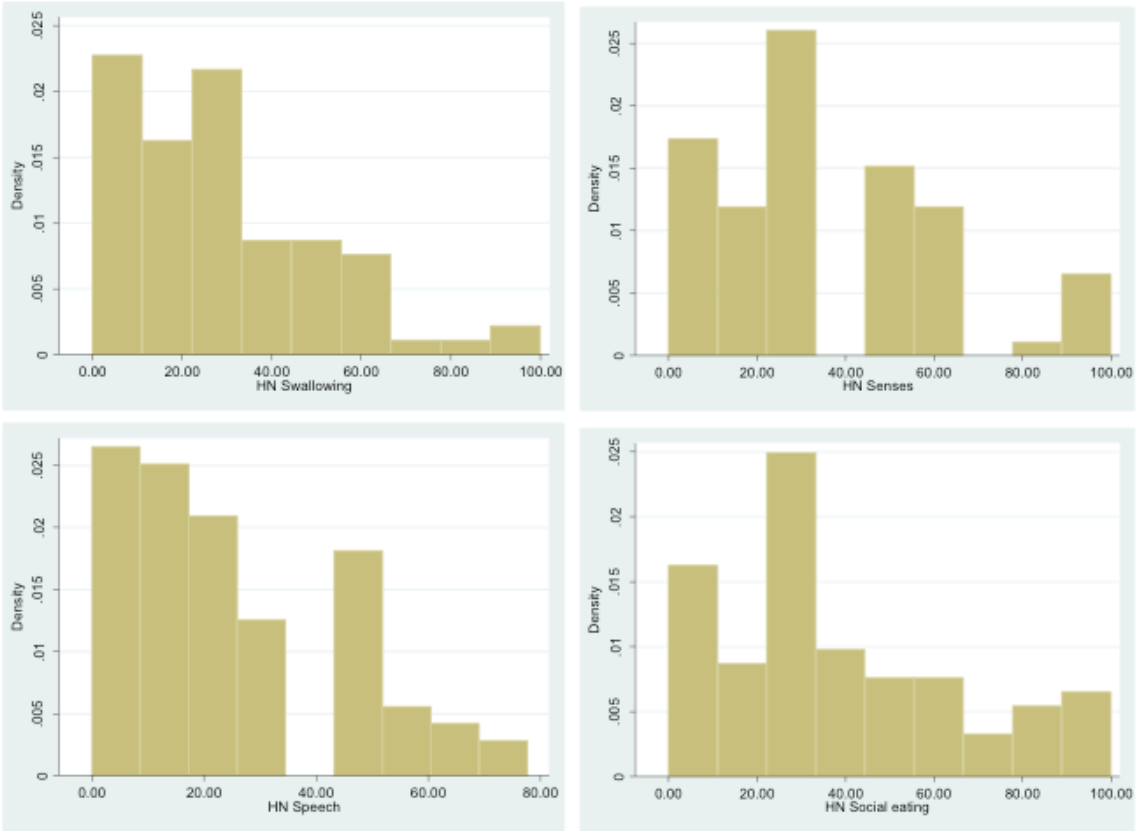


Figure 5-11 Histograms of baseline H&N35 swallowing, sense, speech and social eating scores

The distribution of the mean OH-QoL16 total score at baseline is shown in Figure 5-12.

With regards to the SF 36 questionnaires five sub-scale scores were analysed: general health perception, social role functioning, physical role functioning, mental health, reported health transition (Figure 5-13).

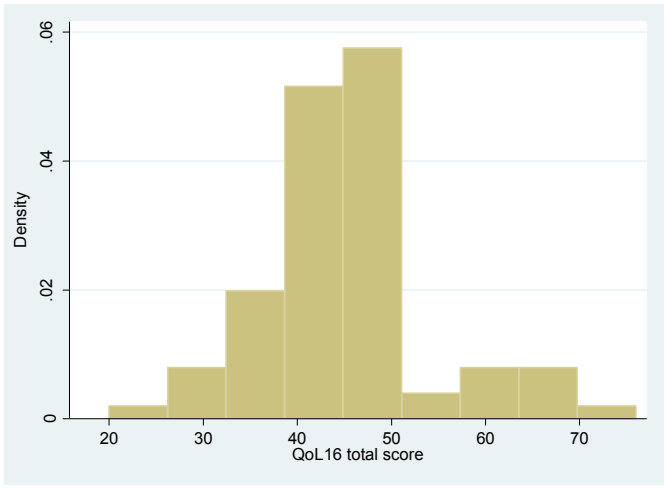


Figure 5-12 Histogram of baseline OH-QoL16 overall score

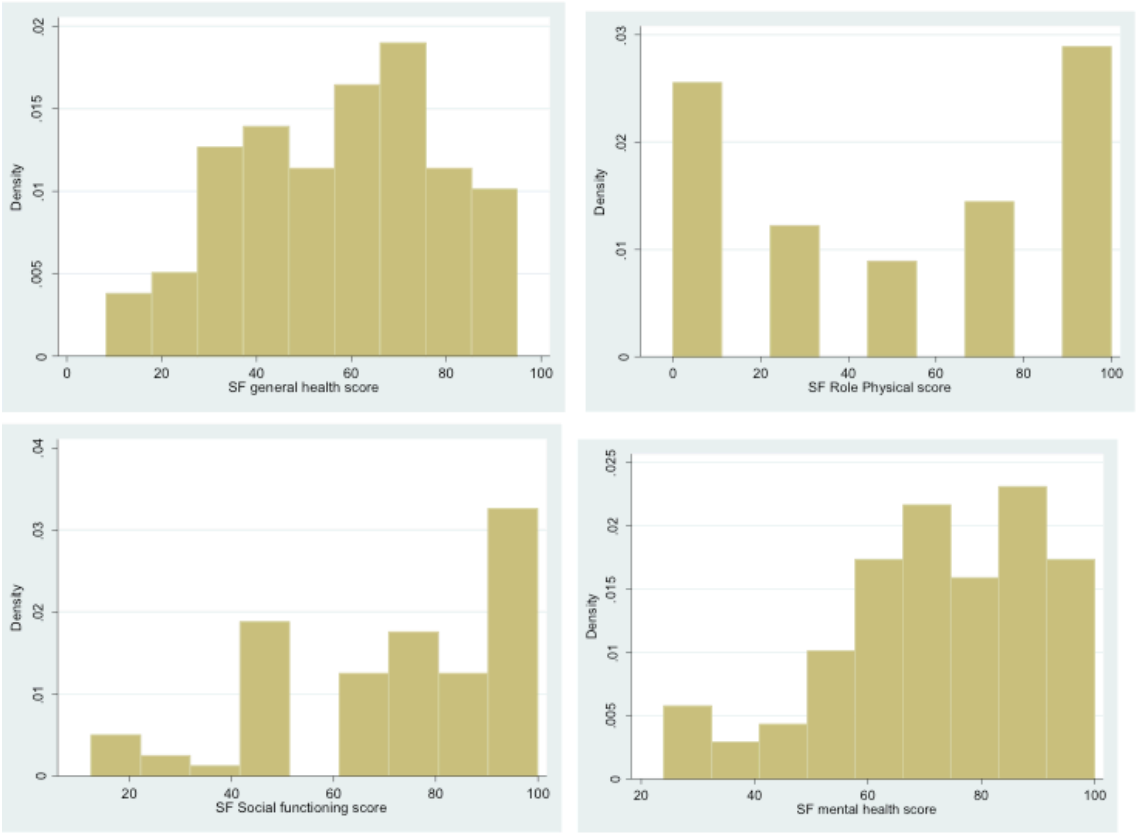


Figure 5-13 Histograms of baseline SF36 scores

5.3 Outcomes analyses

5.3.1 Primary outcome

The primary outcome (reduction of 30% in VAS dry mouth scale from baseline) was met by more participants in the sham group (31%) compared

to the active group (22%). Therefore the null hypothesis that there would be no difference of >30% in the VAS scale between test and control group could not be rejected (Table 5-2).

Table 5-2 Results from analysis of primary outcome

≥ 30% reduction in dry mouth symptoms? (n=67)	Sham (n=36)	Active (n=32)
Yes	11 (31%)	7 (22%)
No	25 (69%)	25 (78%)

Logistic regression models have been fitted to adjust odds ratios for centre, age, gender, type of radiotherapy, years since radiotherapy completion and number of residual salivary glands. The results were not statistically significant and are represented in Table 5-3. Although the statistical analysis plan indicated to adjust the odds ratios also for the dosage of radiotherapy to primary tumour, the analysis was not performed due to the missing data in participants recruited in Bradford Royal Infirmary.

Table 5-3 Logistic regression models for primary outcome

Unadjusted analyses (n=68)	Estimate	95% Confidence interval	
Unadjusted analyses (n=68)			
Difference in proportions	-0.087	(-0.295 to 0.121)	
Odds ratio	0.64	(0.18 to 2.16)	P-value: 0.58 (exact)
Adjusted analysis			
Odds ratio adjusted for centre (n=68)	0.60	(0.20 to 1.83)	P-value: 0.37
Odds ratio adjusted for centre, age, gender (n=68)	0.50	(0.15 to 1.74)	P-value: 0.28
Odds ratio adjusted for centre, age, gender, Type of radiotherapy, years since radiotherapy completion, Number of residual salivary glands (n=64)	0.43	(0.11 to 1.64)	P-value: 0.22

5.3.2 Secondary outcomes

5.3.2.1 Continuous VAS scores

An independent-samples t-test was conducted to compare the mean VAS score at 12 months in the active group and in the sham group, adjusted for the baseline VAS score, centre, gender, type of radiotherapy and residual number of major salivary glands. The histogram presented in Figure 5-14 shows the distribution for this variable.

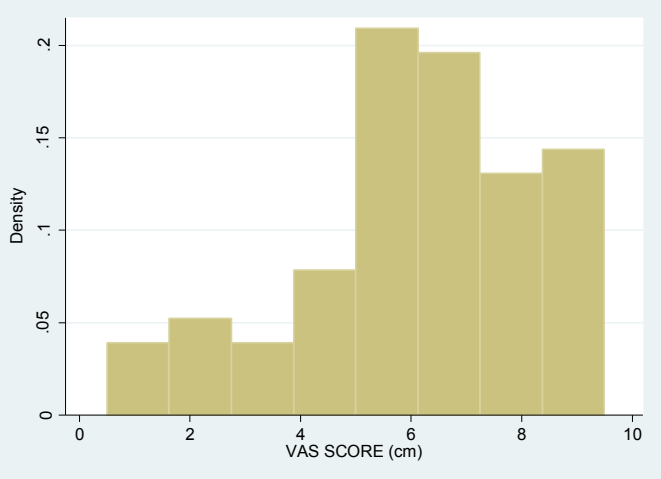


Figure 5-14 Histogram of 12 month VAS scores

There was a not significant difference in the VAS score at 12 months between the active group (M=5.93, SD=2.26) and the sham group (M=6.17, SD=2.03); p = 0.65 (Table 5-4).

The Figure 5-15 summarised the change in the VAS score over 12 months. Improvement seems to be greater in the active group over the first 6 visits (except visit 3), however the treatment effect over the time was not significant (Table 5-5).

Table 5-4 Results from analysis of VAS scores

	Mean (SD) / Median (IQR)		Treatment effect (difference in means)	95% Confidence interval	P-value
	Active (n=32)	Sham (n=36)			
VAS dry mouth symptoms score Mean (SD)	5.93 (2.26)	6.17 (2.03)	-0.23	-1.28 to 0.80	0.65
Adjusted analysis					
Adjusted for baseline VAS (n=68)			-0.18	-1.23 to 0.88	0.74
Adjusted for baseline VAS and centre (n=68)			-0.14	-1.21 to 0.94	0.80
Adjusted for baseline VAS, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=64)			-0.05	-1.13 to 1.03	0.93

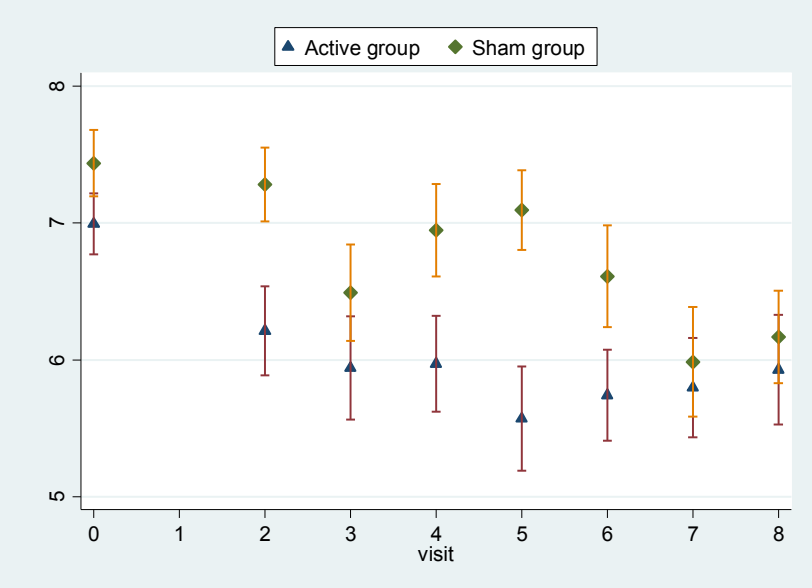


Figure 5-15 Continuous VAS scores over time (mean + SE bars)

Table 5-5 Results from analysis of continuous VAS scores over time (random effects model)

Mean (SD) / Median (IQR)	Treatment effect (difference in means)	95% Confidence interval	P-value
Adjusted analysis			
Adjusted for baseline VAS and visit (n=85)	-0.58	-1.18 to 0.04	0.065
Adjusted for baseline VAS, visit and centre (n=85)	-0.56	-1.17 to 0.05	0.074
Adjusted for baseline VAS, time, centre, age, gender, Type of radiotherapy, years since radiotherapy completion, Number of residual salivary glands, (n=80)	-0.52	-1.16 to 0.12	0.114

5.3.2.2 Salivary flow rate

An independent-samples t-test was conducted to compare the mean unstimulated whole salivary flow rate at 12 months in the active group and in the sham group, adjusted for the baseline unstimulated whole salivary flow rate, centre, gender, type of radiotherapy and residual number of major salivary glands. The histogram presented in Figure 5-16 shows the distribution for this variable.

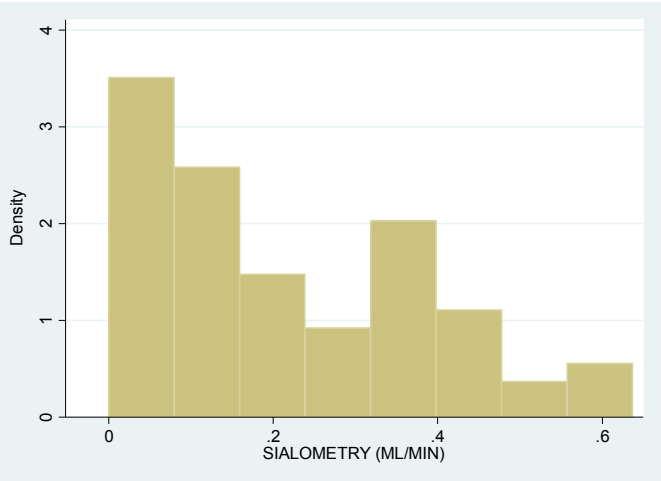


Figure 5-16 Histogram of 12 month salivary flow rate (ml/min)

There was a non significant difference in the whole unstimulated salivary flow score at 12 months between the active group (M=0.22, SD=0.17) and the sham group (M=0.21, SD=0.18); p = 0.78 (Table 5-6).

Table 5-6 Results from analysis of continuous 12 month salivary flow rate

	Mean (SD) / Median (IQR)		Treatment effect (difference in means)	95% Confidence interval	P-value
	Active (n=32)	Sham (n=36)			
Salivary flow rate (ml/min) Mean (SD) median (IQR)	0.22 (0.17) 0.17 (0.07 to 0.36)	0.21 (0.18) 0.15 (0.06 to 0.33)	0.01	-0.07 to 0.09	0.78
Adjusted analysis					
Adjusted for baseline (n=68)			-0.002	-0.08 to 0.08	0.96
Adjusted for baseline and centre (n=68)			-0.0009	-0.08 to 0.08	0.98
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=64)			-0.012	-0.10 to 0.08	0.78

The results for the analysis estimated using a generalised linear model (GLM) assuming a Gamma distribution did not differ fundamentally from the base-case analysis (Table 5-7, 5-8)

Table 5-7 Sensitivity analysis – GLM with identity link and gamma distribution

	Mean (SD) / Median (IQR)		Treatment effect (difference in means)	95% Confidence interval	P-value
	Active (n=32)	Sham (n=36)			
Salivary flow rate (ml/min) Mean (SD) median (IQR)	0.22 (0.17) 0.17 (0.07 to 0.36)	0.21 (0.18) 0.15 (0.06 to 0.33)	0.01	-0.07 to 0.09	0.78
Adjusted analysis					
Adjusted for baseline (n=68)			-0.01	-0.09 to 0.06	0.66
Adjusted for baseline and centre (n=68)			-0.02	-0.09 to 0.06	0.64
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=64)			-0.03	-0.11 to 0.04	0.39

Table 5-8 Sensitivity analysis – regression using square root transformation

	Mean (SD)		Treatment effect	95% Confidence interval	P-value
	(transformed scale)		**(transformed scale)**		
	Active (n=32)	Sham (n=36)			
Square root of Salivary flow rate (ml/min) Mean (SD)	0.43 (0.19)	0.41 (0.21)	0.027	-0.07 to 0.13	0.59
Adjusted analysis					
Adjusted for baseline (n=68)			0.0007	-0.09 to 0.09	0.99
Adjusted for baseline and centre (n=68)			0.002	-0.09 to 0.09	0.97
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=64)			-0.007	-0.10 to 0.09	0.88

The Figure 5-17 summarised the change in the whole unstimulated salivary flow rate over 12 months. The improvement seems to be greater in the active group over the treatment (except visit 6), however the treatment effect was not significant (Table 5-9).

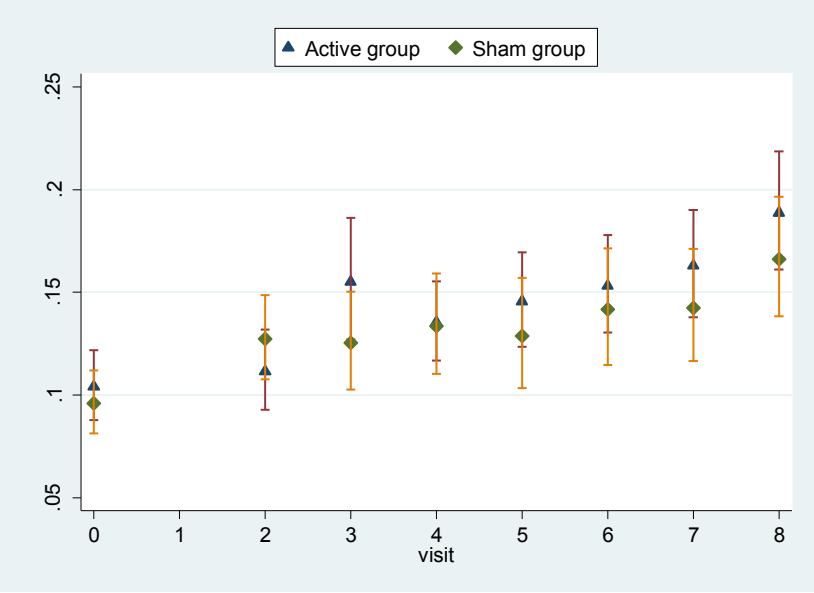


Figure 5-17 Continuous salivary flow rate over time

Table 5-9 Results from analysis of salivary flow rate over time (random effects model)

	Treatment effect (difference in means)	95% Confidence interval	P-value
Adjusted analysis			
Adjusted for baseline (n=85)	-0.005	-0.053 to 0.042	0.83
Adjusted for baseline and centre (n=85)	-0.005	-0.053 to 0.042	0.83
Adjusted for baseline, centre, age, gender, Type of radiotherapy, years since radiotherapy completion, Number of residual salivary glands, (n=80)	-0.016	-0.066 to 0.033	0.52

5.3.2.3 EORTC QLQ-H&N35

The results of the EORTC QlQ-H&N35 questionnaires with respects to the multi-item subscales showed a lower score in the active group at 12 months for the domains swallowing, senses, speech, social contact and sexuality. In the social eating and pain domain the difference was in favour of the control group (Table 5-10).

Table 5-10 Summary data for QLQ-H&N35 domain scores at 12 months: mean (SD) and median (interquartile range).

Domain score	Active (n=31)	Sham (n=36)
Pain	23.66 (16.96) 25.00 (8.33 to 33.33)	21.76 (23.93) 12.50 (0 to 33.33)
Swallowing	20.52 (20.57) 16.67 (8.33 to 25)	23.23 (22.14) 16.67 (8.33 to 41.67)
Senses	28.89 (28.34) 16.67 (0 to 50) (n=30)	32. 87 (25.35) 33.33 (16.67 to 50.00)
Speech	17.92 (21.02) 11.11 (0 to 22.22)	18.83 (21.71) 11.11 (0 to 33.33)
Social Eating	29.84 (26.94) 25.00 (8.33 to 41.67)	29.09 (27.12) 16.67 (8.33 to 41.67)
Social Contact	11.40 (21.85) 0 (0 to 13.33)	11.94 (22.67) 0 (0 to 6.67)
Sexuality	21.84 (32.76) 0 (0 to 33.33) (n=29)	23.04 (28.13) 8.33 (0 to 33.33) (n=34)

The analyses of the EORTC H&N-35 single items (Table 5-11) showed that fewer participants in the active group had quite a bit or very much problems with mouth opening (5 vs. 11) and quite a bit or very much dry mouth (18 vs. 26). The results were similar for the item sticky saliva (17), cough (6 vs. 7), problems with teeth, use of feeding tube (2) and weight gain (5). More participants in the active group have lost weight (6 vs. 3), used pain killers (14 vs. 13) and taken nutritional supplements (7 vs. 4) but less felt ill (2 vs. 4).

Table 5-11 Summary data for QLQ-H&N35 domain scores at 12 months – those based on single questionnaire items

Domain		Active (n=31)	Sham (n=36)
Teeth: have you had problems with your teeth?	Not at all	15 (48%)	21 (58%)
	A little	9 (29%)	7 (20%)
	Quite a bit	4 (13%)	5 (14%)
	Very much	3 (10%)	3 (8%)
Opening mouth: have you had problems opening your mouth wide?	Not at all	13 (42%)	13 (36%)
	A little	13 (42%)	12 (34%)
	Quite a bit	4 (13%)	8 (22%)
	Very much	1 (3%)	3 (8%)
Dry Mouth: Have you had a dry mouth?	Not at all	1 (3%)	0
	A little	12 (39%)	10 (28%)
	Quite a bit	9 (29%)	14 (39%)
	Very much	9 (29%)	12 (33%)
Sticky Saliva: Have you had sticky saliva? (n=64)	Not at all	2 (7%)	8 (23%)
	A little	11 (37%)	10 (29%)
	Quite a bit	13 (43%)	13 (37%)
	Very much	4 (13%)	4 (11%)
Cough: Have you coughed? (n=65)	Not at all	10 (33%)	14 (39%)
	A little	14 (47%)	15 (42%)
	Quite a bit	5 (17%)	5 (14%)
	Very much	1 (3%)	2 (5%)
Felt Ill: Have you felt ill?	Not at all	23 (74%)	24 (67%)
	A little	6 (19%)	8 (22%)
	Quite a bit	2 (7%)	4 (11%)
	Very much	0	0
Pain killers: Have you used pain killers? (n=65)	No	16 (53%)	23 (64%)
	Yes	14 (47%)	13 (36%)
Nutritional supplement: Have you taken nutritional supplements?	No	24 (77%)	32 (89%)
	Yes	7 (23%)	4 (11%)
Feeding tube: Have you used a feeding tube?	No	29 (94%)	34 (94%)
	Yes	2 (6%)	2 (6%)
Weight loss: Have you lost weight?	No	24 (80%)	33 (92%)
	Yes	6 (20%)	3 (8%)
Weight gain: Have you gained weight?	No	26 (84%)	30 (86%)
	Yes	5 (16%)	5 (14%)

The Figure 5-18 shows the histograms of 12-month domain score for the multi-item scales assessed (swallowing, senses, speech and social eating).

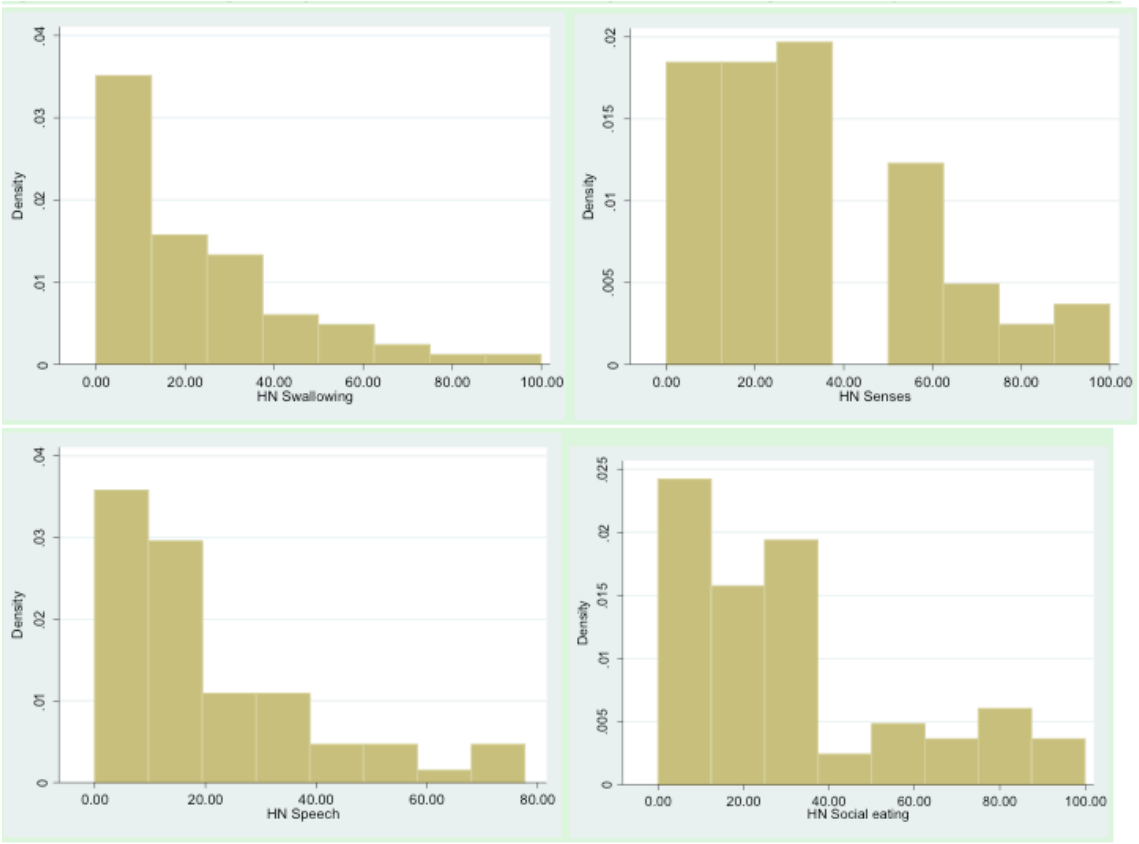


Figure 5-18: Histogram of 12 month H&35 domain scores for swallowing, senses, speech, social eating

An independent-samples t-test was conducted to compare the mean score for the domains swallowing, sense, speech and social eating and the single items of the EORTC QoL-H&N35 questionnaires at 12 months in the active group and in the sham group, adjusted for the baseline values, centre, gender, type of radiotherapy and residual number of major salivary glands. There was a non significant difference in means with regards to the swallowing domain (-2.71; 95% CI -13.19 to 7.78), sense (-3.98; 95% CI -17.19 to 9.23), speech (-0.91; 95% CI -11.38 to 9.56), social eating (0.75; 95% CI -12.48 to 13.98) (Table 5-12).

Table 5-12 Comparison of swallowing, senses, speech, social eating scores

	Treatment effect (difference in means)	95% Confidence interval	P-value
Swallowing domain scores			
Unadjusted analysis (N=67)	-2.71	-13.19 to 7.78	0.61
Adjusted for baseline (n=66)	-3.95	-12.83 to 4.93	0.38
Adjusted for baseline and centre (n=65)	-3.98	-12.95 to 4.99	0.38
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=61)	-6.04	-14.97 to 2.89	0.18
Senses domain scores			
Unadjusted analysis (n=66)	-3.98	-17.37 to 9.43	0.55
Adjusted for baseline (n=64)	-4.27	-13.90 to 5.36	0.38
Adjusted for baseline and centre (n=64)	-4.20	-13.92 to 5.52	0.39
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=60)	-4.91	-15.32 to 5.49	0.35
Speech domain scores			
Unadjusted analysis (n=67)	0.91	-11.38 to 9.56	0.86
Adjusted for baseline (n=66)	1.26	-6.45 to 8.96	0.75
Adjusted for baseline and centre (n=66)	1.29	-6.49 to 9.07	0.74
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=61)	-0.01	-8.02 to 7.99	0.99
Social eating domain scores			
Unadjusted analysis (n=67)	0.75	-12.48 to 13.98	0.91
Adjusted for baseline (n=66)	2.74	-7.31 to 12.79	0.59
Adjusted for baseline and centre (n=66)	2.81	-7.34 to 12.96	0.58
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=61)	1.69	-8.49 to 11.86	0.74

There was no significant difference in the treatment effects also for the single item of the H&N35 questionnaire dry mouth, sticky saliva and cough (Table 5-13).

Table 5-13 Comparison of dry mouth, sticky saliva, coughing scores between randomised groups at 12 months

	Odd ratio	95% Confidence interval	P-value
Dry mouth score			
Unadjusted analysis (N=67)	0.65	0.27 to 1.59	0.35
Adjusted for baseline score (n=66)	0.45	0.17 to 1.21	0.11
Adjusted for baseline score and centre (n=66)	0.45	0.17 to 1.20	0.11
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=62)	0.38	0.13 to 1.15	0.09
Sticky saliva score			
Unadjusted analysis (n=65)	1.63	0.66 to 3.99	0.29
Adjusted for baseline score (n=63)	1.61	0.61 to 4.20	0.33
Adjusted for baseline score and centre (n=63)	1.60	0.61 to 4.19	0.34
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=60)	1.55	0.55 to 4.39	0.40
Cough score			
Unadjusted analysis (n=66)	1.18	0.48 to 2.92	0.72
Adjusted for baseline score (n=65)	1.04	0.40 to 2.74	0.93
Adjusted for baseline score and centre (n=65)	1.07	0.41 to 2.82	0.89
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=61)*	1.01	0.36 to 2.83	0.99

The Figures 5-19, 5-20, 5-21 and 5-22 summarised the change in the subscales swallowing, sense, speech, and social eating over the time. An improvement can be observed over time for all the analysed subscale, less pronounced for the sense subscale score and not statically significant (Table 5-14). However, analysis of repeated measurements showed a statistically

significant improvement over time for the dry mouth and swallowing item of the EORTC-QoL-H&N35 questionnaire.

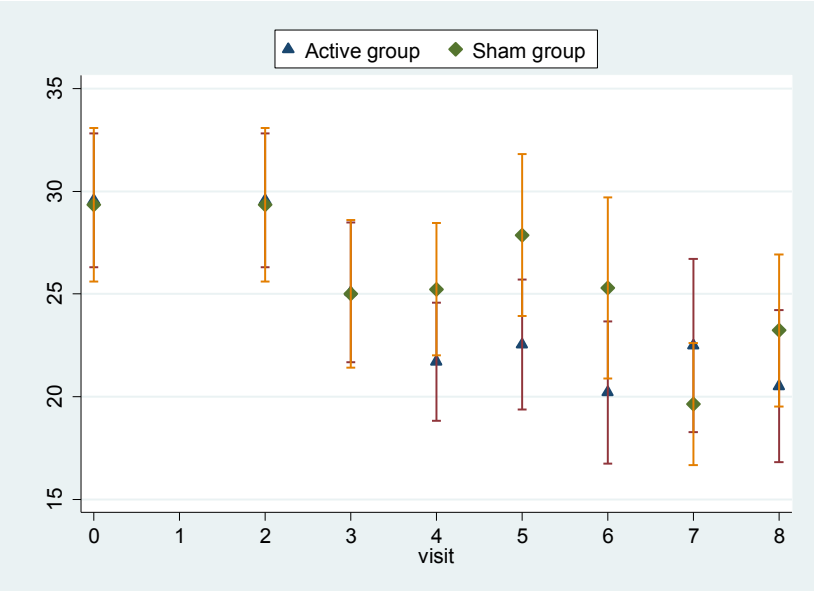


Figure 5-19 H&N35 swallowing sub-scale scores over time

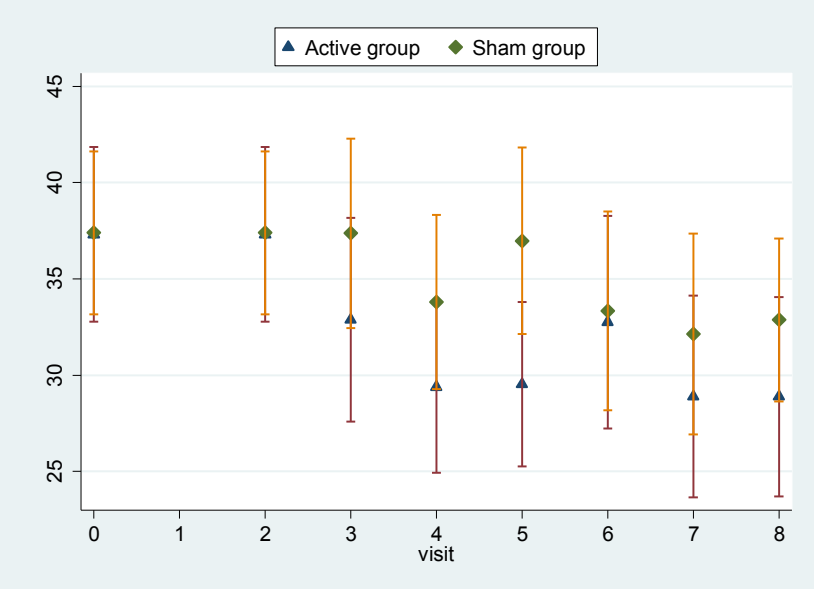


Figure 5-20 H&N35 sense subscale scores over time

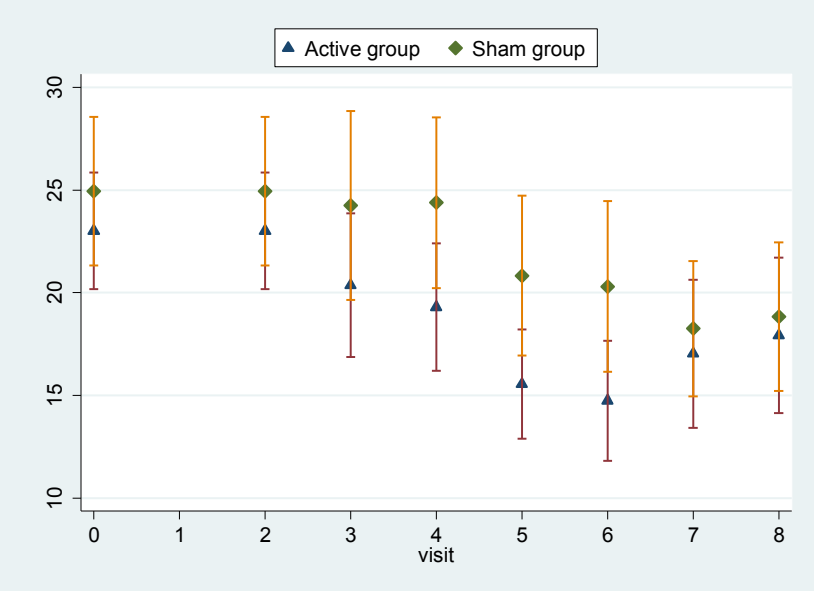


Figure 5-21 H&N35 speech sub-scale scores over time

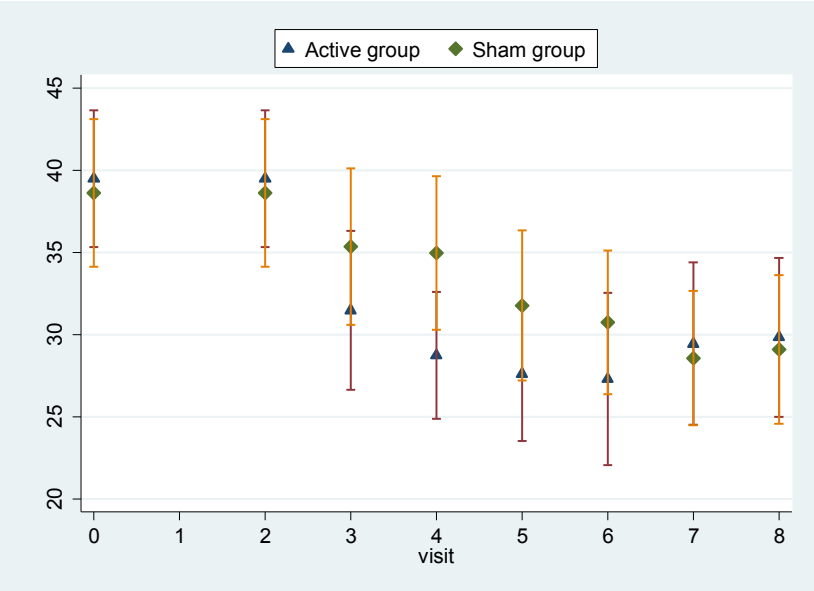


Figure 5-22 H&N35 social eating sub-scale scores over time

Table 5-14 Results from analysis of H&N scores over time (random effects models)

	Treatment effect (difference in means)	95% Confidence interval	P-value
Swallowing domain scores			
Adjusted for baseline score (n=83)	-3.48	-8.00 to 1.05	0.13
Adjusted for baseline score and centre (n=83)	-3.46	-7.96 to 1.04	0.13
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)	-4.54	-9.16 to 0.07	0.05
Senses domain scores			
Adjusted for baseline score (n=83)	-2.38	-7.66 to 2.90	0.38
Adjusted for baseline score and centre (n=83)	-2.38	-7.67 to 2.92	0.38
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)	-2.25	-7.98 to 3.48	0.44
Speech domain scores			
Adjusted for baseline score (n=83)	0.68	-4.33 to 2.97	0.72
Adjusted for baseline score and centre (n=83)	-0.68	-4.31 to 2.95	0.71
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)	-1.43	-5.37 to 2.50	0.47
Social eating domain scores			
Adjusted for baseline score (n=83)	-1.94	-7.03 to 3.13	0.46
Adjusted for baseline score and centre (n=83)	-1.94	-7.06 to 3.18	0.46
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)*	-3.09	-8.52 to 2.34	0.27

	Odd ratio	95% Confidence interval	P-value
Dry mouth score			
Adjusted for baseline score (n=83)	0.52	0.22 to 1.21	0.13
Adjusted for baseline score and centre (n=83)	0.52	0.22 to 1.21	0.13
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)	0.47	0.22 to 1.01	0.05
Sticky saliva score			
Adjusted for baseline score (n=82)	1.39	0.72 to 2.68	0.33
Adjusted for baseline score and centre (n=78)	1.39	0.72 to 2.68	0.33
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=60)	1.27	0.64 to 2.50	0.50
Cough score			
Adjusted for baseline score (n=83)	0.72	0.34 to 1.55	0.40
Adjusted for baseline score and centre (n=83)	0.72	0.34 to 1.55	0.40
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)	0.72	0.32 to 1.60	0.42

5.3.2.4 OH-QoL16

An independent-samples t-test was conducted to compare the mean OH-QoL16 overall score at 12 months in the active group and in the sham group, adjusted for the baseline overall score, centre, gender, type of radiotherapy and residual number of major salivary glands. The histogram presented in Figure 5-23 shows the distribution for this variable.

There was a non significant difference in the OH-QoL16 overall score at 12 months between the active group (M=46.83 SD=10.68) and the sham group (M=48.75, SD=10.19); p = 0.46 (Table 5-15).

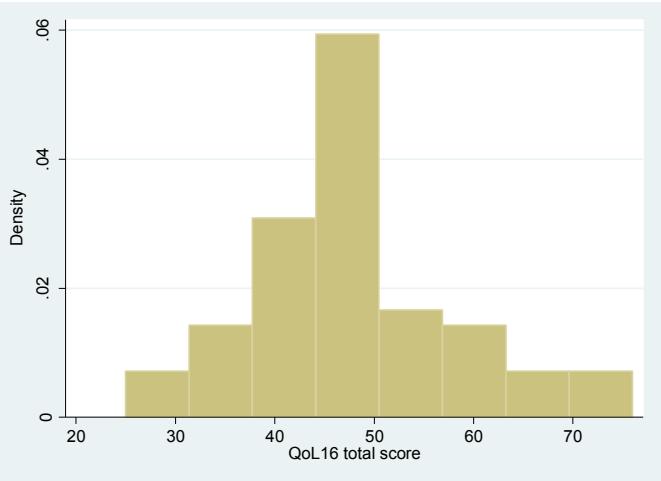


Figure 5-23 Histogram of 12 months OH-QoL overall score

Table 5-15 Results from analysis of continuous 12 months OH-QoL questionnaire (overall score)

	Mean (SD) / Median (IQR)		Treatment effect in (difference means)	95% Confidence interval	P-value
OH-QoL16 (Overall Oral health score)	46.83 (10.68)	48.75 (10.19)	-1.92	-7.06 to 3.23	0.46
Adjusted analysis					
Adjusted for baseline score and centre (n=64)			-3.68	-7.58 to 0.21	0.06
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=64)			-3.15	-7.01 to 0.72	0.11

There was a trend toward a higher OH-QoL overall score (all good effects of extreme impact) over the treatment, but the difference seems greater in the sham group compared to the active group (Figure 5-24; Table 5-16).

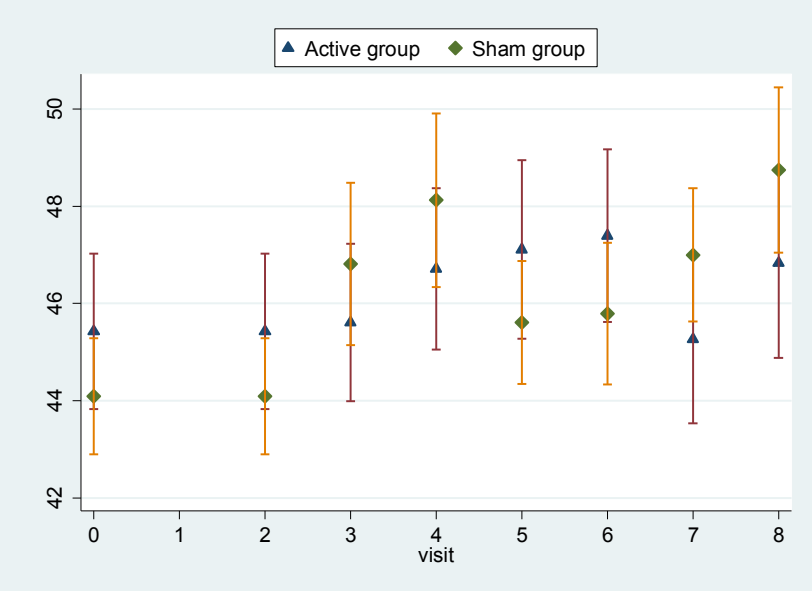


Figure 5-24 OH-QoL16 overall score over time

Table 5-16 Results from analysis of OH-QoL16 overall score over time (random effects model)

	Treatment effect (difference in means)	95% Confidence interval	P-value
Adjusted analysis			
Adjusted for baseline score (n=81)	-1.53	-3.49 to 0.42	0.12
Adjusted for baseline score and centre (n=83)	-1.54	-3.50 to 0.42	0.12
Adjusted for baseline score, centre, age, gender, Type of radiotherapy, years since radiotherapy completion, Number of residual salivary glands, (n=76)	-1.30	-3.38 to 0.78	0.22

5.3.2.5 SF-36

An independent-samples t-test was conducted to compare the mean SF-36 subdomains of general health, physical role functioning, social role functioning, mental health and reported health transition. The histogram presented in Figure 5-25 shows the distribution for these variable.

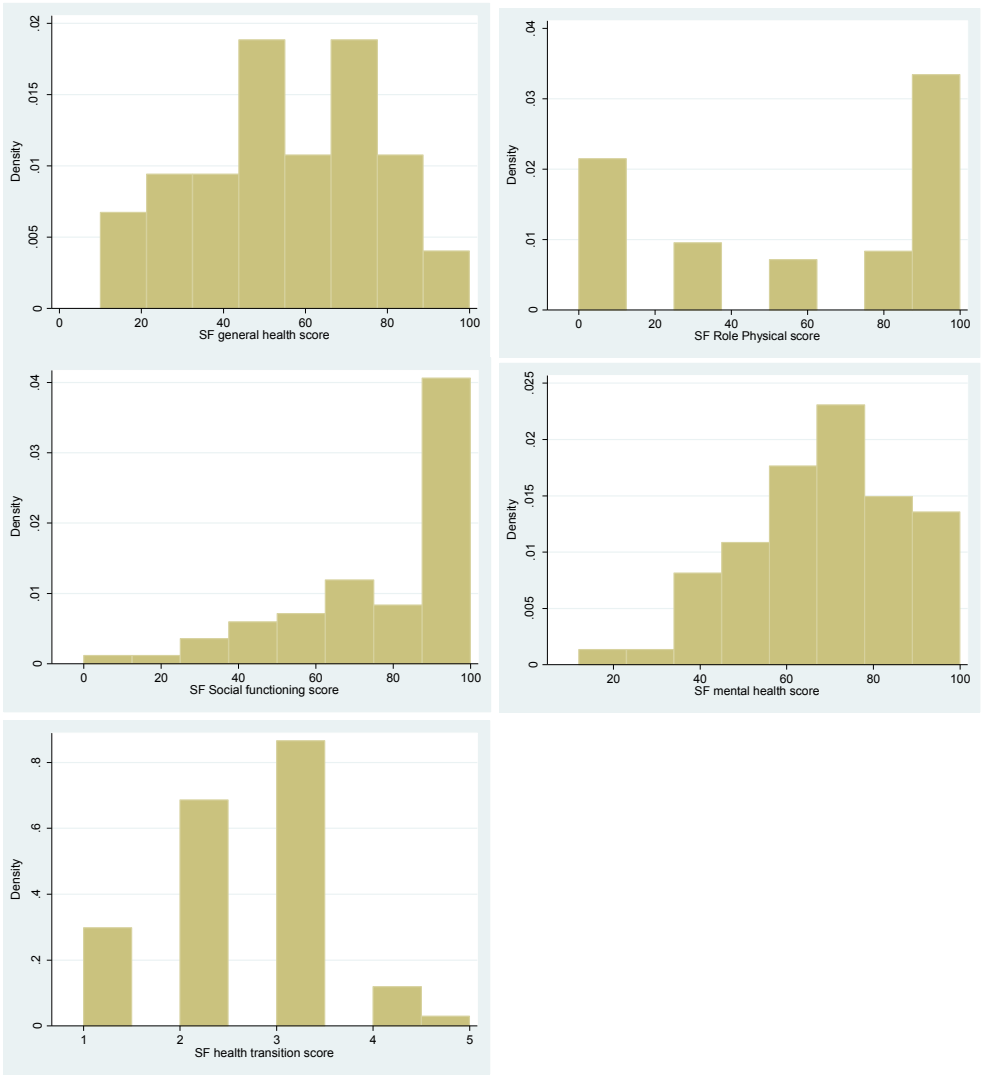


Figure 5-25 Histogram of 12 month SF-36 domain scores for general health, physical role, social role, mental health, reported health transition.

The subdomains were adjusted for the baseline values, centre, gender, type of radiotherapy, and residual number of major salivary glands.

There was a non significant difference in the general health subdomain and the social role functioning score at 12 months between the active group (M=52.75, SD=23.74) and the sham group (M=59.11, SD=21.00) and (M=77.02 SD=24.17) and the sham group (M=72.57, SD=28.32); p = 0.50, respectively. The difference was also not significant for mental health subdomain for the active group (M=65.58 SD=16.77) compared to the sham

group (M=69.89 SD=19.82) (p = 0.35) and reported health transition (p = 0.56).

The difference was non significant for the physical role functioning in the active group (M=60.48 SD=45.08) and the sham group (M=54.63, SD=40.89); p = 0.58 (Table 5-17).

Table 5-17 Comparison of general health, physical role functioning, social functioning, mental health and reported health transition between randomised groups at 12 months

	Mean (SD) / Median (IQR)		Treatment effect (difference in means)	95% Confidence interval	P-value
General Health domain scores					
	Active (n=31)	Sham (n=35)			
Mean (SD) median (IQR)	52.75 (23.74) 52 (35 5o 72)	59.11 (21.00) 57 (45 to 77)	-6.36	-17.37 to 4.64	0.25
Adjusted analysis					
Adjusted for baseline and centre (n=65)			-5.13	-13.51 to 3.24)	0.23
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=61)			-4.48	-13.64 to 4.68	0.33
Physical Role Functioning domain scores					
	Active (n=31)	Sham (n=36)			
Mean (SD) median (IQR)	60.48 (45.08) 75 (0 to 100)	54.63 (40.89) 50 (12.5 to 100)	5.85	-15.13 to 26.84	0.58
Adjusted analysis					
Adjusted for baseline and centre (n=65)			1.25	-18.01 to 20.52	0.90
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=61)			2.35	-19.11 to 23.81	0.83
Social functioning domain scores					
	Active (n=31)	Sham (n=36)			
Mean (SD) median (IQR)	77.02 (24.17) 87.5 (50 to 100)	72.57 (28.32) 75 (62.5 to 100)	4.45	-8.51 to 17.41	0.50
Adjusted analysis					
Adjusted for baseline and centre (n=66)			3.43	-6.46 to 13.32	0.49
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=60)			5.75	-4.95 to 16.45	0.29
Mental health					

	Active (n=31)	Sham (n=36)			
Mean (SD) median (IQR)	65.58 (16.77) 65 (52 to 76)	69.89 (19.82) 72 (58 to 86)	-4.31	-13.35 to 4.73	0.35
Adjusted analysis					
Adjusted for baseline and centre (n=66)			-2.90	-10.07 to 4.27	0.43
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=62)			-0.75	-7.84 to 6.33	0.83
Reported Health Transition					
	Active (n=31)	Sham (n=36)			
Mean (SD) median (IQR)	2.52 (0.73) 3 (2 to 3)	2.39 (0.99) 2 (2 to 3)	0.13	-0.30 to 0.56	0.56
Adjusted analysis					
Adjusted for baseline and centre (n=66)			0.18	-0.26 to 0.62	0.42
Adjusted for baseline, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=62)			0.04	-0.42 to 0.51	0.19

There was a trend toward a higher general health perception score, physical role functioning and health transition score over time in the sham group, with a lower mental health score and unchanged social functioning. Participants in the active group showed a lower general health perception and mental health score at 12 months compared to baseline, an unchanged physical role functioning and a higher score for the domains reported health transition and social functioning (Figures 5-26, 5-27, 5-28, 5-29, and 5-30). The difference in mean of the reported mental health transition between the two groups was statistically significant (Table 5-18).

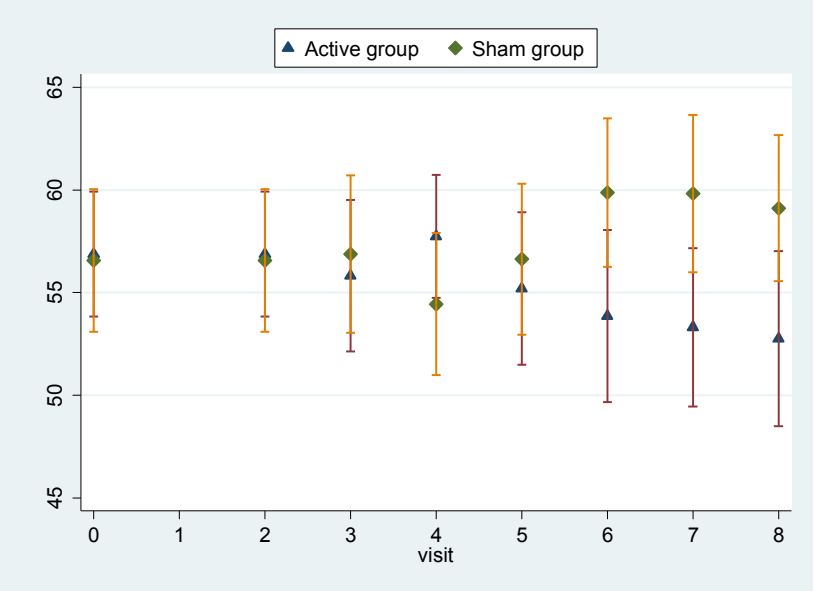


Figure 5-26 SF-36 General health perception score over time

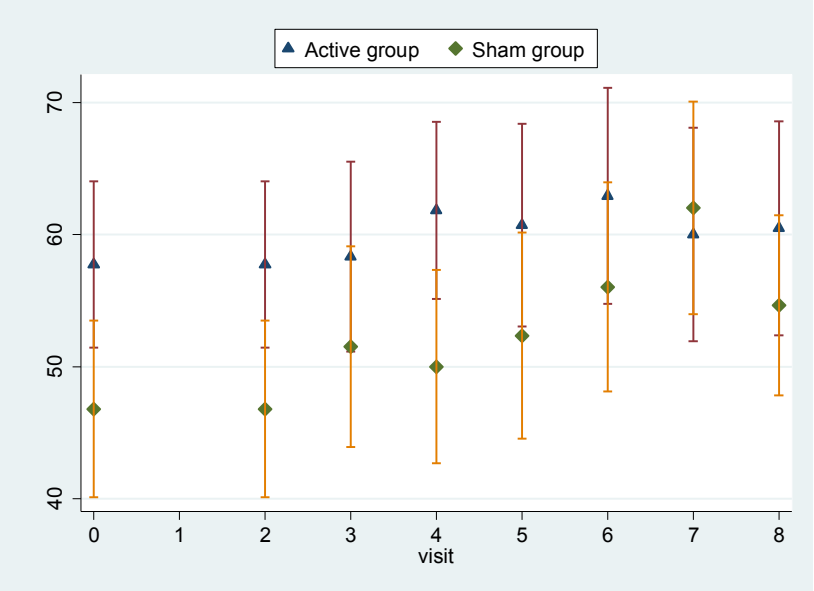


Figure 5-27 SF-36 Physical role functioning score over time

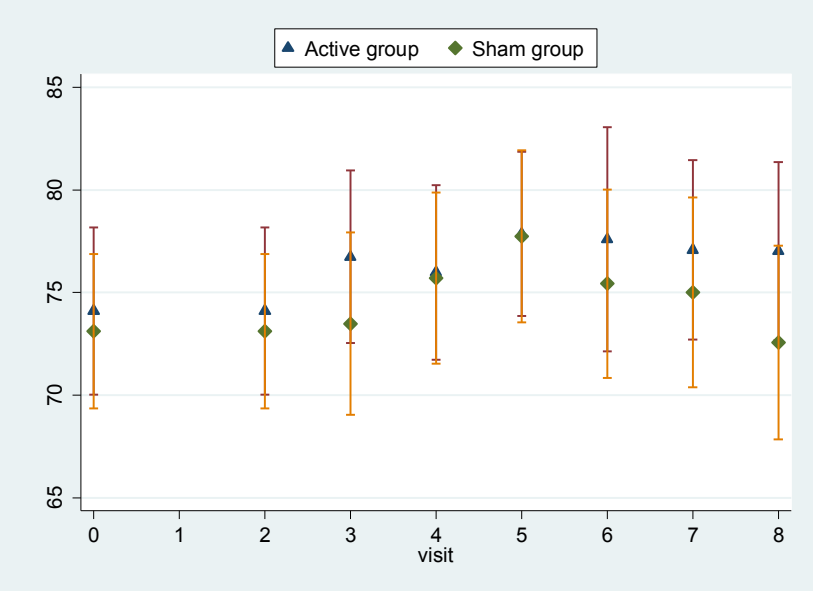


Figure 5-28 SF-36 Social role functioning over time

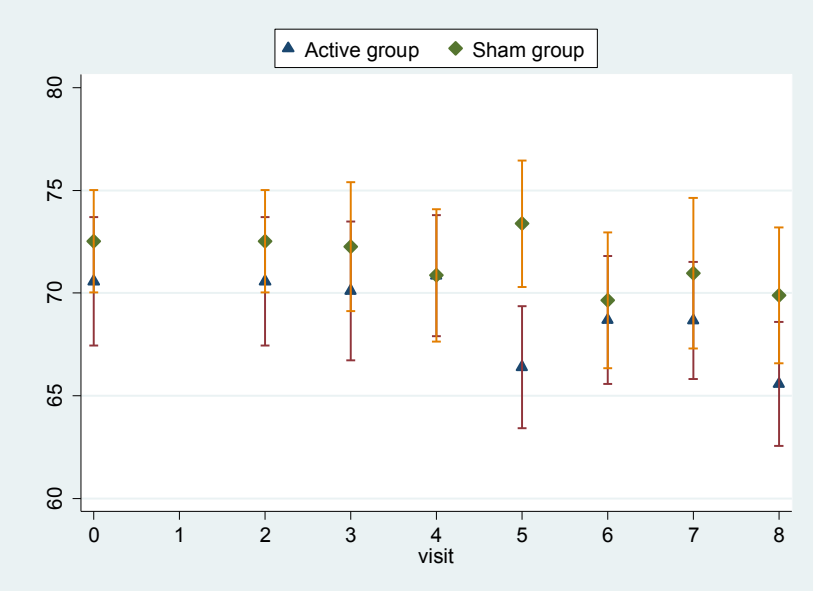


Figure 5-29 SF-36 Mental Health score over time

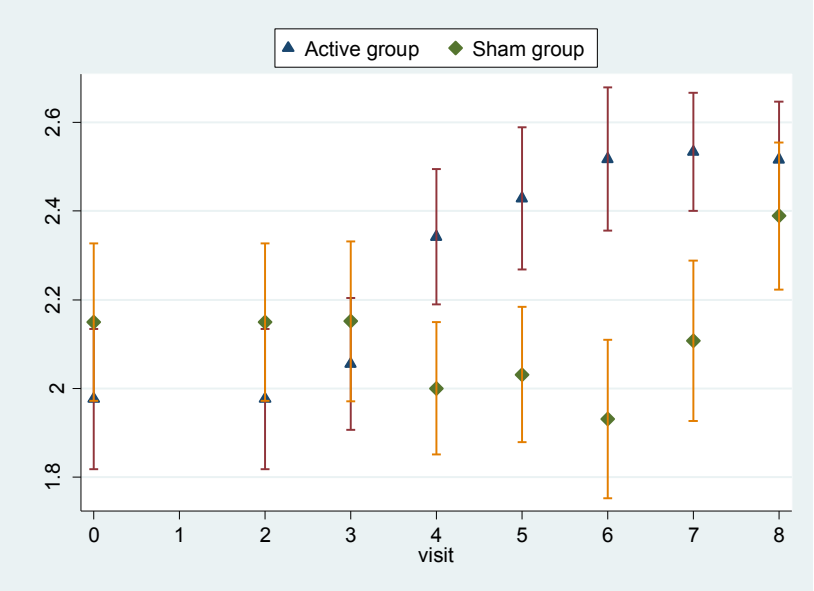


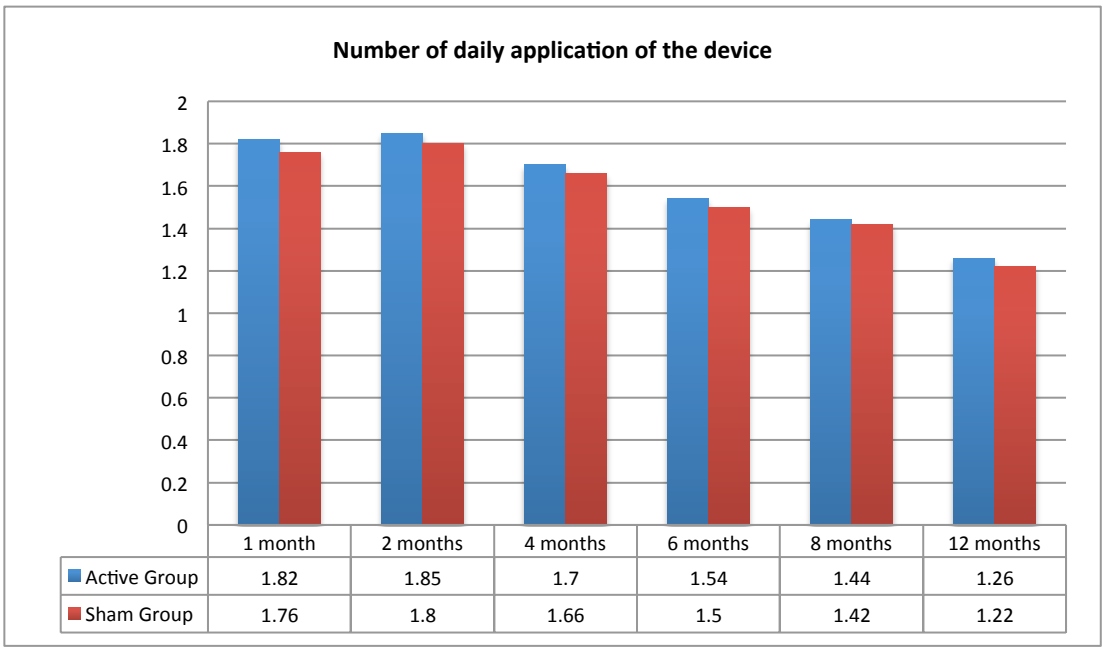
Figure 5-30 SF-36 Reported Health Transition score over time

Table 5-18 Results from analysis of SF-36 scores over time (random effects models)

	Treatment effect (difference in means)	95% Confidence interval	P-value
General Health perception			
Adjusted for baseline score (n=82)	0.40	-3.99 to 4.79	0.86
Adjusted for baseline score and centre (n=82)	0.42	-4.01 to 4.84	0.85
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=7)	-0.006	-4.82 to 4.80	0.99
Role Physical Score			
Adjusted for baseline score (n=81)	3.30	-6.13 to 12.73	0.49
Adjusted for baseline score and centre (n=81)	3.31	-6.20 to 12.82	0.50
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=76)	3.34	-7.46 to 14.14	0.54
Social functioning			
Adjusted for baseline score (n=83)	1.14	-3.60 to 5.88	0.64
Adjusted for baseline score and centre (n=83)	1.14	-3.63 to 5.92	0.64
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=78)	2.03	-3.12 to 7.18	0.44
Mental Health			
Adjusted for baseline score (n=82)	-1.04	-4.38 to 2.30	0.54
Adjusted for baseline score and centre (n=82)	-1.07	-4.39 to 2.25	0.53
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=77)	0.47	-3.94 to 3.00	0.79
Health Transition			
Adjusted for baseline score (n=82)	0.31	0.09 to 0.53	0.005
Adjusted for baseline score and centre (n=82)	0.31	0.10 to 0.53	0.005
Adjusted for baseline score, centre, age, gender, type of radiotherapy, years since radiotherapy completion, number of residual salivary glands (n=77)	0.22	0.002 to 0.43	0.05

5.3.2.6 Device usage

The average number of applications used by patients per day was 1.79 per day during the first month, with an average daily cumulative use of 8.9 minutes. The number of applications declined over the study period, as it can be seen in the Figure 5-31.



	1 month	2 months	4 months	6 months	8 months	12 months
Active Group	1.82	1.85	1.7	1.54	1.44	1.26
Sham Group	1.76	1.8	1.66	1.5	1.42	1.22

Figure 5-31 Average daily number of device application

In the present study participants' compliance with regards to appointment attendance was similar between the active and sham group (Figure 5-32).

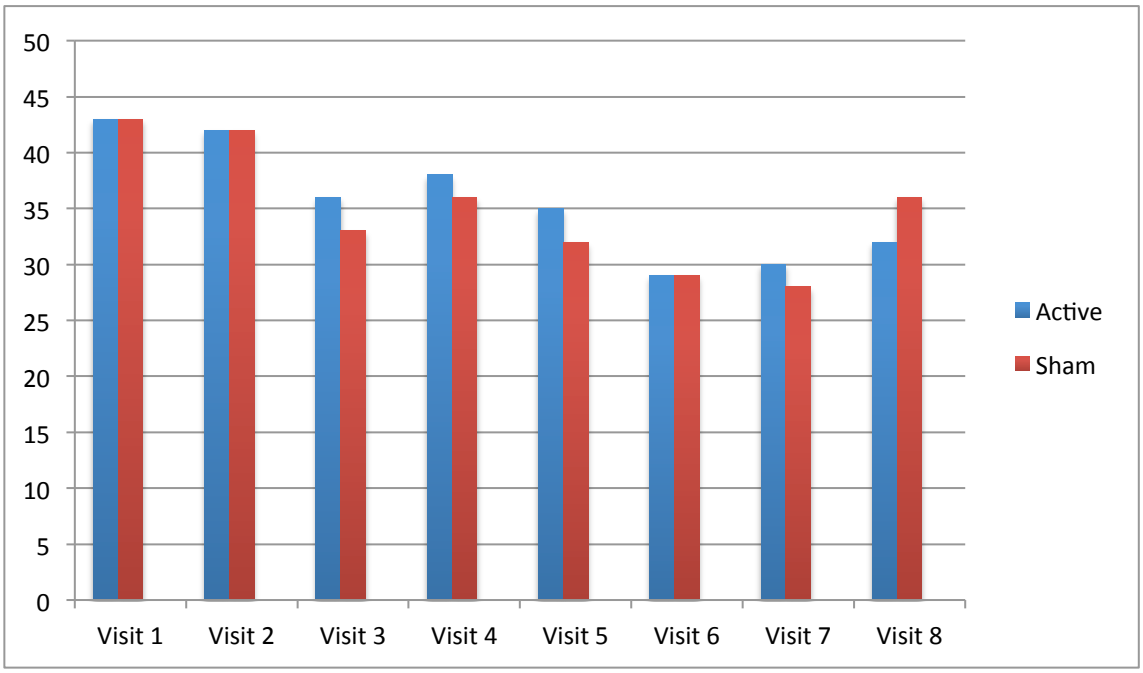


Figure 5-32 Attendance Rate of Participants

The study was completed by 68 participants (58 at UCL and 10 in Bradford) of the 84 receiving the allocated intervention. Thirty-six participants in the sham group and thirty-two in the active group completed the study. The overall dropout rate was 19%, as expected in the sample size calculation.

Reasons given by patients for not completing the study are displayed in Table 5-19. Time constraints and personal problems were an issue in 7 participants. As expected recurrence of cancer was the reason for the exclusion of 3 participants from the trial (one oesophageal cancer, one lung cancer, one tumour to the base of the spine). Other reasons for dropout were being unable to use the device due to PEG tube feeding (1 participant), to dental pain (1 participant), to bone exposure (1 participant) and device not fitting properly and causing discomfort (3 participants).

Table 5-19 Drop-out rate and reasons

Reasons for Drop-out		Number of patients
Withdrawal of consent	UCLH Bradford	4 3
Cancer	UCLH Bradford	3 0
Unable to use the device due to PEG tube feeding	UCLH Bradford	0 1
Unable to use the device due to dental pain	UCLH Bradford	0 1
Unable to use the device due to bone exposure	UCLH Bradford	0 1
Unable to use the device due to discomfort	UCLH Bradford	0 3

We recorded six Serious Adverse Event, related to 4 admissions to the hospitals for reason not related to the study (continuous abdominal pain, hernia repair surgery, ankle surgery, swollen right arm) and one osteonecrosis of the jaw. One participant presented to his second review appointment with a broken investigational device: the two 2-mm electrodes were missing, likely broken during intraoral usage. It remains unclear when the event happened, as the patient was unaware of the episode and denied respiratory or gastrointestinal symptoms.

For the theoretical potential risk of inhalation/ingestion and consequent damage to respiratory or GI tract, a chest plus abdominal X-ray has been arranged but was unable to locate the electrodes. The participant completed the trial with no complications.

Due to the long duration of the study we had 36 non-serious advent events, the most common being cold, fungal infections, oral ulcerations and dental extractions (Table 5-20).

Table 5-20 Non-serious advent events

Side effects			
Serious Adverse Event		UCLH	4
		Bradford	2
	Admission to hospital		4
	Osteonecrosis		1
	Potential inhalation/ingestion of the device electrodes		1
Non Serious Adverse Event		UCLH	33
		Bradford	3
	Anaemia		1
	Broken wrist		3
	Burning sensation		1
	Cold		4
	Dizziness		1
	Dysphagia		2
	Ear infection		3
	Fungal infection		4
	Gingivitis		1
	Hernia		1
	Hypertension		1
	Kidney mass		1
	Migraine		1
	Muscle spasms		1
	Nasal obstruction		1
	Oral keratosis		1
	Oral pain		1
	Oral ulcer		5
	Parotid gland swelling		1
	Pigmented lesion		1
	Root canal treatment		1
	Swelling buccal mucosa		1
	Throat infection		3
	Tooth extraction		4
	Tooth fracture		1

6 Discussion

Head and neck cancers represent 7% of all cancers that are diagnosed in the world, with approximately 600,000 new cases per year (Ferlay et al., 2012). Nearly two-thirds of all HNC patients will receive radiation as part of their treatment. IMRT has been shown to reduce the incidence of xerostomia. Nevertheless at 12 months approximately forty per cent of patients would still report xerostomia of grade 2 or worse. Therefore, even with IMRT, nearly 200,000 HNC cancer survivors will develop persistent xerostomia, with significant reduction in their quality of life due to the impact of dry mouth symptoms upon daily activities, speech, swallowing, sleeping, and emotional function (Kakoei et al., 2012).

Our systematic review has demonstrated that pilocarpine can provide some benefit in patients with radiotherapy-associated xerostomia. This medication, however, is not well-tolerated and up to 20% of patients discontinue the treatment with pilocarpine because of the low benefit-to-cost ratio, both in terms of side effects and efficacy of the treatment (Rieke, 1995).

Salivary electrostimulators are emerging devices that can apply electrical stimuli to the submucosal/subcutaneous cranial nerve that innervate the salivary glands, thus stimulating salivation. Among them, the remote controlled intraoral removable device GenNarino (Saliwell Ltd, Saarbruecken, Germany) has emerged for its miniaturized size and user-friendliness. Saliwell GenNarino has been granted approval for marketing in Europe (CE mark) and is sold at a price of $575 (£370/500€) per device (Sasportas et al., 2013), thus significantly less than 12 months of pilocarpine therapy at maximum dosage (£1096).

The aim of this clinical trial was to assess the long-term potential benefit of the removable intra-oral electrostimulating device GenNarino in the management of radiotherapy-induced xerostomia.

The results of this RCT showed a reduction in VAS dry mouth symptoms of 1.27 for participants assigned to the non-active device providing only mechanical stimulation and 1.06 for participants assigned to the active devices providing electrical and mechanical stimulation. Statistical analyses showed that the difference between the two means was not significant ($P>0.05$). Therefore the main null hypothesis of this RCT could not be rejected. In other words the trial failed to show any significant difference in the VAS score (dry mouth) between cases and controls. There was a trend towards to an improvement in the VAS score over time but the difference between the two groups was not significant.

With regards to the secondary outcomes, the mean salivary flow rate in patients receiving the active device was higher compared to the patients using the sham device but the difference between the two means was not statistically significant ($P>0.05$).

The analyses of Quality of Life questionnaires revealed a benefit, although non-significant, for the domains swallowing, senses, speech and social eating of the EORTC H&N-35 single items in patients using the active device. The Oral Health Quality of life questionnaires did not reveal any benefit in the quality of life of participants allocated to the active group.

The SF-36 showed higher score of physical role functioning and social functioning (indicating better functioning) and reported health transition in the active group but lower score in the general health perception and mental

health. The treatment effect, however, was not significant for any subscale. Therefore the hypothesis for the secondary outcomes also could not be rejected.

The results of this clinical trial conflict with the findings of other clinical trials showing a preferential short-term effect for the active second-generation electrostimulating device compared to the sham device.

However, our study differs significantly from previous studies.

The only two previous placebo-controlled studies, showing an improvement of the salivary function following electrostimulation device, used the first generation device (Salitron) and did not include any patient with radiotherapy-induced xerostomia (Steller et al., 1988, Talal et al., 1992). One study testing the first generation of electrostimulator device included irradiated patients and the results of the studies were stratified for this condition, however the study design was open-label and uncontrolled.

Quantitative comparison of LEONIDAS-2 clinical trial with other studies on the second generation electrostimulating device is problematic because of the lack of uniformity in study design and definition of endpoints. Previous trials excluded participants with history of radiation to the head and neck, and if included, they represented only a small proportion of the included population. Furthermore the results were never stratified for patients with radiotherapy-induced xerostomia and so they cannot be entirely compared to the results of this study.

This is the only study limiting the recruitment to patients with radiotherapy-associated xerostomia. One clinical trial on first generation electrostimulating

device have included, among other causes of xerostomia, patients with radiotherapy-associated xerostomia (13 out of 24) (Weiss Jr et al., 1986). Two clinical trials on second generation electrostimulating devices included 14 and 9 patients respectively (12% and 7% of the total cohort) (Strietzel et al., 2011, Alajbeg et al., 2012).

The first proof-of-concept clinical trials on the II generation device (GeNnarino) by Strietzel et al excluded patients with depression, complete edentulous patients and irradiated patients (Strietzel et al., 2007). Included patients were tested with one active stimulation and one sham stimulation of 10 minutes in a random order with a crossover design. Objective evaluation included a wetness sensor whereas the subjective sensation was recorded asking the participants if the effect of both experiments was similar or one of them had a better effect. After 1 min of wearing GeNnarino, the registered dryness status was similar in the two groups, whereas after an application of 3, 5 and 10 minutes a lower dryness could be observed in the active mode. Subjectively 24% of the patients did not perceive any differences between the two tests and a difference was only perceived in 33% of participants when the active device was used after the sham but not when used before. Quality of life has not been assessed in this study.

Four years later the same author designed a clinical trial which this time included also patients with radiotherapy-associated xerostomia (Strietzel et al., 2011). Patients were excluded if they had mental disease or depression and were not allowed to use systemic sialogogues only during the first two months of the trial, which had a total duration of 11 months. In the first stage of the study, participants were randomised to use the active and the sham

device for one month each. Participants were asked to use the device for 10 minutes each application and the average daily cumulative length of use of the electrostimulation device was 40 minutes. In the LEONIDAS-2 study each application was limited to 5 minutes and the average daily cumulative use was significantly lower (8 minutes). The active intervention performed better than sham with regards to subjective VAS xerostomia scale, with a change of 8 mm in the VAS scale vs. 5 mm (P=0.05), but not with regards to stimulated or unstimulated salivary flow. Although the different study design (cross-over), duration (2 months) and lack of stratification of the results for irradiated patients make comparison with our study difficult, one possible explanation of the different treatment effect could also be the higher length of device use.

The study was followed by an open-label phase, during which the active devices were used for additional 9 months, completed only by 60% of the initial cohort and only 7% of them with our condition of interest (Alajbeg et al., 2012). The average daily cumulative use of the device was 18 minutes in this study, with applications ranging from 1 to 10 minutes. There was no control in this study and a statistically significant difference in the VAS scale of 12 mm has been observed between month 11 and baseline. The median difference in unstimulated salivary flow between endpoint and baseline was 0.062 ml/min ($p<0.05$). In our study the median difference after 12 months was similar (0.08) with no difference among the active and sham group. The difference in the quality of life was not statistically significant and results are not comparable because a different and not validated QoL questionnaire has been used in the study.

The participants in this study were recruited from three Head and Neck Cancer Unit in UK (University College London Hospital, The Royal Marsden Hospital, Bradford Royal Infirmary). Participants were also referred from the Guy's and St Thomas' NHS Foundation Trust. The setting is different from other studies on salivary gland electrostimulation, where patients were recruited in the Sjogren's syndrome Clinic (Steller et al., 1988), in an Oral Medicine or Oral Surgery Department (Strietzel et al., 2007) or referred by the Rheumatologist (Strietzel et al., 2011).

In all previous trials the vast majority of participants were women (81%-86%)(Strietzel et al., 2011, Alajbeg et al., 2012, Strietzel et al., 2007, Talal et al., 1992, Steller et al., 1988), whereas in our study 80% of the participants were men. Men and women may differ in their response to treatment for several reasons, including differences in the size of salivary glands. Menopause and hormone replacement therapy do not seem be associated with salivary dysfunction of the parotid (De Almeida et al., 2008).

The mean age of 58 years was comparable to the other studies (Strietzel et al., 2011, Alajbeg et al., 2012, Strietzel et al., 2007, Steller et al., 1988).

The salivary flow and saliva composition are strictly related to the presence of systemic disease. Xerostomia can be due to autoimmune exocrinopathies (notably Sjogren's syndrome), dehydration, cognitive alteration, oral sensory dysfunction and psychological disorders. Metabolic disturbances, alteration of the gland innervation as seen in neurological disorders can also cause hyposalivation and/or alter salivary composition. In this study, patients with Sjogren's syndrome were excluded; however, xerostomic medical conditions were present in 23% of the sham and 14% of the active group. These

included diabetes (7 participants), hypothyroidism (4), anxiety (1), and depression (3).

Xerostomia is a relatively common complaint, notably in poorly controlled diabetics due to a decreased flow rate of both unstimulated and stimulated salivary. A change in salivary composition with regards to antimicrobial substances, protein and buffering capacity has also been observed in patients with diabetes (Vissink et al., 2015).

Unstimulated and stimulated salivary flow is decreased in patients with hypothyroidism and autoimmune thyroiditis. In addition salivary gland enlargement (sialadenosis) has also been described. Furthermore anxiety and depression are well recognized causes of reduced salivary flow (Scarabelot et al., 2014).

More than 500 medications are associated with xerostomia. The drugs most commonly implicated in xerostomia are tricyclic antidepressants, antipsychotics, benzodiazepines, atropinics, β-blockers, and antihistamines, due to the anticholinergic or sympathomimetic action of the cited medication. In our study 33% of participants in the sham group and 30% in the active group were taking xerostomic medications.

In the LEONIDAS-2 study the majority of patients had oropharyngeal cancer (53% in the sham group vs. 57% in the active group), which is often cured with radiotherapy. Although parotid-sparing intensity-modulated radiation therapy is now a standard radiotherapy technique for patients with HNSCC, there are no randomised trials evaluating the submandibular-sparing technique. The submandibular glands contribute for 70% to the resting salivary flow, producing saliva rich in mucins. In oropharyngeal cancer

patients, it is rarely possible to spare the ipsilateral submandibular gland, due to the close proximity to the primary tumour. In this study submandibular glands received an average dose of 57 Gy and the dose was higher in the active group (60 Gy). Regarding the dose response, Murdoch-Kinch et al. reported that submandibular gland-stimulated salivary function decreased significantly after a mean dose of >40 Gy (Murdoch-Kinch et al., 2008). This threshold are compatible with a review of the literature reporting a lesser sensitivity of the submandibular glands compared to the parotid glands, for which the threshold is 26 Gy.

Adherence to the treatment is a main factor for success in therapeutic approach and the lower daily cumulative use of the device in this study compared to the other clinical trials could also have affected the results. It is very important, therefore, to analyse the reasons that can have influenced the patient's compliance with the device. The main reason for lack of use of the device was device failures, when the device could not have been switched on. In case of device failure, a first attempt was made to change the batteries on the remote control. In the event of a permanent failure, the manufacturer was contacted in order to request a new device. Device replacement was required for 10 participants. While waiting for the replacement, which took an average of 8 weeks, participants were asked to use the device as normal.

Serious adverse events were not related to the intervention of the study, whereas expected non-serious adverse events were oral fungal infections, one episode of osteonecrosis and oral ulcers.

The risk of fungal infections is largely increased in patients with salivary gland hypofunction due to the loss of the antimicrobial actions (Porter et al., 2004). Bone necrosis secondary to radiation damage was reported as early as 1926, with an identifiable trauma in 65% of the cases (Marx, 1983). The occurrence of oral ulcers in the study (5%) is in keeping with the prevalence of ulcerations in the general population, between 5% and 25% (McCullough et al., 2007).

Although the study did not meet the planned main outcome of dry mouth symptoms reduction in the active device arm versus sham device and there was no evidence of significantly increased salivary function, we have observed a statistically significant reduction in dry mouth symptoms and swallowing perception over time. These results suggest that some benefits may be expected with the use of electrostimulation although there remains at the moment no convincing evidence from our trial.

We suggest that further research is needed and that future studies should consider defining a better phenotyped and more homogenous study population and testing the revised stimulating device, which is characterised by easier fitting that is not operator-dependent.

Study limitations

LEONIDAS-2 clinical trial's primary outcome was defined as the proportion of participants reporting a 30% reduction of xerostomia symptoms after 12 months of treatment, as evaluated through a 100mm VAS. A critical analysis has been performed during the study to estimate the magnitude of change of the visual analogue scale in head and neck settings, by looking at randomized clinical trial that used the visual analog scale to assess

radiotherapy-induced xerostomia. Results showed that the average change scores in controlled clinical trials of treatment for radiotherapy-induced xerostomia was lower than the one estimated in the protocol, being between 20% and 25%. Furthermore we have identified that the magnitude of VAS change (effect size) following the intervention could be more informative for decision-makers than the dichotomous presentation. We have therefore added the analysis of continuous VAS as a secondary outcome of the study.

Participants recruited in this study were heterogeneous with respect to ethic group, smoking habits, site and stage of primary tumour, years since radiotherapy completion. Heterogeneity occurs in many clinical fields and may lead to imbalance of randomized groups in terms of results, even when with strict inclusion criteria. This imbalance, however, has been reduced considerably by following a proper randomization procedure.

A multi-centre design has been chosen to provide evidence that the trial results were not strongly setting-dependent and they could be applied to a broader population of clinical sites. A pilot or feasibility study on a smaller number of patients has not been conducted to clarify issues such as participant recruitment and retention strategies, appropriate length of follow-up and attrition likelihood.

The clinical trial's accrual period has been estimated considering the accrual rate of each clinical centre, using questionnaires to measure the total number of cases currently or recently seen for the targeted condition. Although the accrual period was 6 months longer than planned in order to enforce similarity of centre sample sizes, the planned samples sizes was achieved but the ratio within-centre was unequal, with 79% of the participants recruited

in one centre (University College London Hospital) and 21% in the remaining participating centre (Bradford Royal Infirmary).

Furthermore, the multi-centre design has not allowed us to perform all the statistical analyses detailed in the statistical analysis plans. In particular radiation dose of primary tumour and number of remaining salivary glands were not available for the participants recruited at the Bradford Royal Infirmary.

An important limit of our statistical analysis was that parotid mean dose was not available for participants recruited in both centre and we could not stratify participants based on the parotid mean dose. There is strong evidence that chronic salivary dysfunction is correlated to the mean parotid gland dose and severe xerostomia is usually avoided if at least one parotid gland is spared to a mean dose of less than ≈20 Gy or if both glands are spared to less than ≈25 Gy. Due to the missing data we are unable to comment on whether participants who received a lower parotid mean dose reported better function or higher benefit following the experimental intervention.

Site visits combined with scheduled meetings were arranged between the investigators and the clinical trial coordinator, in order to discuss the deviation of trial recruitment from the expected trajectory graph of recruitment. Problems with recruitment were attributed to strict inclusion and exclusion criteria, mainly in the ability of showing that salivary flow rate could be enhanced by stimulation. Inadequate staffing has also been an issue in the first phase of the trial, later solved by recruiting a new investigator.

The dropout rate was similar to the rate estimated in the sample size. However, of the 70 participants required to satisfy the sample size

requirement, only 68 completed the trial. The attrition rate was significantly lower in one centre (University College London Hospital), where retention strategies such as letter remainders for follow-up study visits, detailed report of the up-to-date status of every participant and calls to reschedule missed study visits were put in place. University College London Hospital also offers the facility of a dedicated clinical research space and team, which includes a trial coordinator. It is possible that the retention strategies and facility played a role in retention. In accordance with the Code of Federal Regulations (the compensation is neither coercive nor at the level that would present undue influence), financial incentives were offered to participants as an incentive for trial completion, enhancing participants' engagement with the trial. Dropout occurred at random and was not related to the randomization assignment.

The large number of follow-up visits, which were eight in total over one year period, has probably increased the likelihood of attrition. In this study 7 participants withdrew their consent because of the time commitment. In order to address this issue, an intention to treat analyses has been performed, including all randomised study participants in the groups to which they were randomised.

Performing statistical analysis with reduced number of patients has an effect on the outcome. With a small number of patients there is a higher standard error and therefore a wider confidence interval. It is therefore more difficult to obtain significant results. This may explain why our results were not statistically significant.

7 Conclusions

In conclusion, the use of a second-generation intra-oral electrostimulating device in this study appeared to be safe, acceptable but not superior to a sham device in relieving dry mouth symptoms, increasing salivary gland function and improving quality of life of head and neck cancer survivor.
There was, however, a tendency of improvement of dry mouth symptoms and swallowing symptoms over time in individuals using the active device.

These results suggest that some benefits may be expected with the use of electrostimulation although there remains at the moment no convincing evidence from our trial. We suggest that further research is needed which may also benefit from some of the information and data obtained in LEONIDAS-2 study in order to inform the design and better phenotyped and more homogenous study population.

A new trial using the recently developed revised version of the electrostimulating device which is characterised by easier fitting that is not operator-dependent, thus overcoming some of the weaknesses of the previous device, can be suggested.

8 Appendix

8.1 Intra-oral electrostimulating device

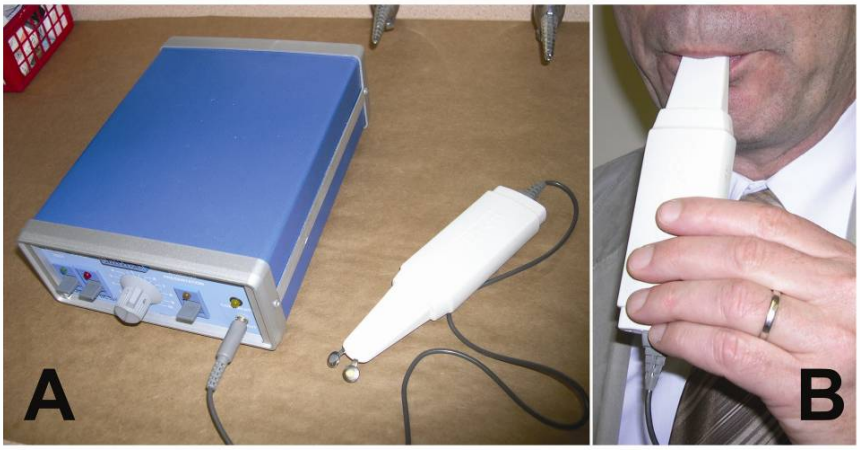


Figure 8-1 First generation electrostimulating device, consisting of a console (A) and a hand-held probe (B)

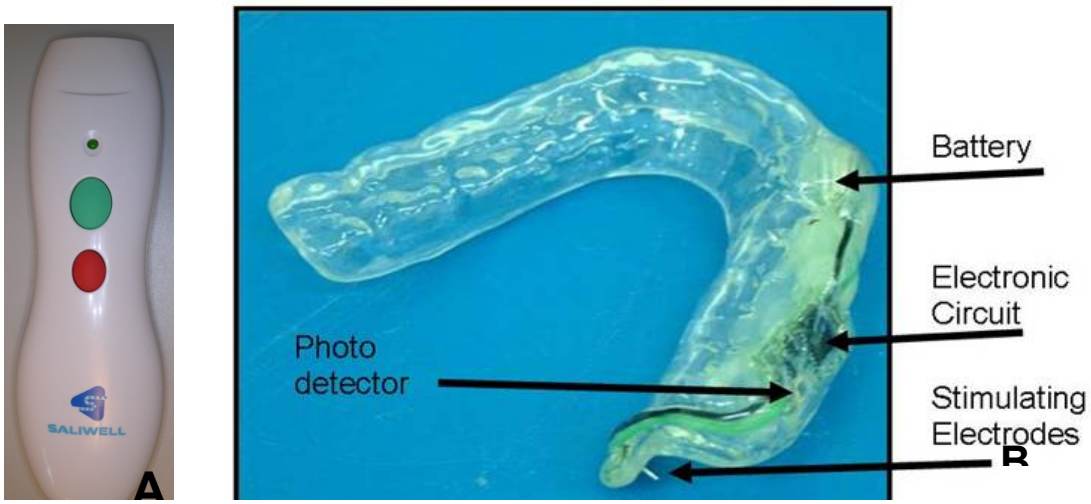


Figure 8-2 Second generation electrostimulating device (Saliwell GeNarino), consisting of a remote control (A) and a removable intraoral device (B)

Figure 8-3 Second generation electrostimulating device (Saliwell GenNarino)

Figure 8-4 Second generation electrostimulating device activation

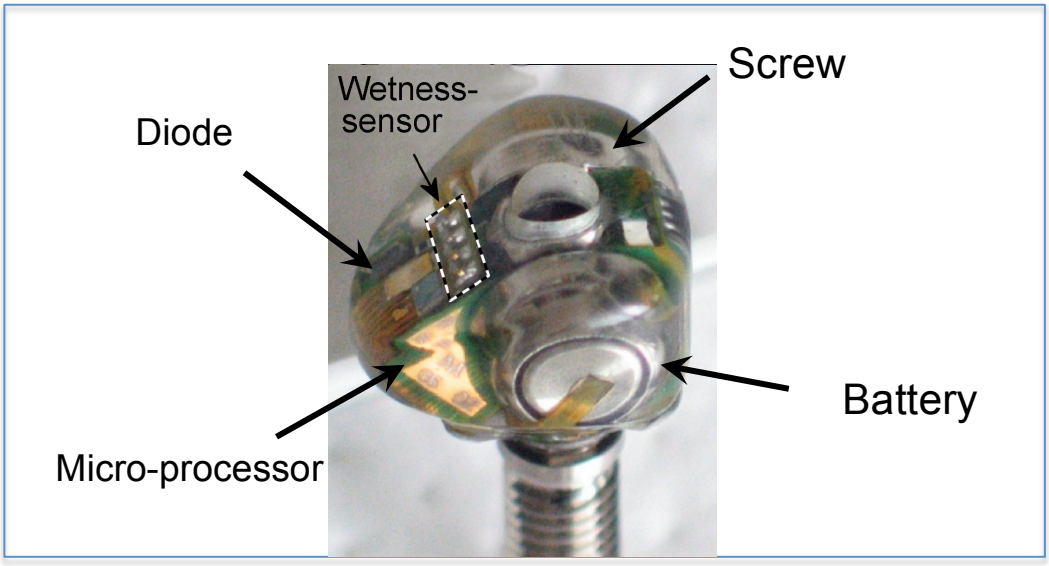


Figure 8-5 Third generation electrostimulating device (Saliwell Crown)

8.2 Participant Information Sheet And Consent Form

Version 6; 28th November 2012
Study Number: OM-11-03
Subject Initials: _____
Screening Number: _____
Randomisation Number (if applicable):_____

STUDY ON A NOVEL MEDICAL DEVICE FOR THE TREATMENT OF REDUCED SALIVATION (DRY MOUTH) RESULTING FROM RADIATION THERAPY

Please read this sheet carefully. Please ask if you do not understand or would like more information

1.Invitation to participate

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have.
You have been selected as a potential participant because you might have the appropriate condition that we are studying. The following information is provided so that you can make an informed decision regarding your willingness to participate. Please discuss with family and friends and ask us if there is anything which is not clear or if you would like more information.

2.What is the purpose of the study?

The majority of patients with cancer of the head and neck (e.g. cancer of the mouth or throat) receive radiotherapy as part of their treatment. Radiotherapy to the head and neck often causes permanent damage to the salivary glands and consequent reduction in salivation – also known as dry mouth. Dry mouth can be very distressing and affect the way you talk and eat. Unfortunately, current therapies of dry mouth are often unsatisfactory, and may result in adverse side effects. Preliminary studies indicated that a device that releases mild and painless electric stimuli to skin of the mouth can increase salivation and reduce the sensation of dry mouth.
The main purpose of this study is to investigate whether this device will reduce dry mouth symptoms in patients who had radiotherapy to the Head and Neck.
The medical device to be investigated in this research was introduced few years ago, showed to be effective and safe in humans and was recently granted approval for commercialization in EU.
We also aim at analysing and comparing levels of salivary components associated by the device as it has been suggested that electrostimulation may also alter the composition of saliva and the function of cells that secret saliva.

3. Why have I been invited?

You have been identified as a potential participant by doctors in your cancer or dental clinic because you have persistent dry mouth due to radiotherapy to the Head and Neck.

4. Do I have to take part?

It is up to you to decide to join the study. We will describe the study and go through this information sheet, and you can take as much time as you need to decide upon taking part in this study. If you agree to take part, we will then ask you to sign a

consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive. Participation in this study will in no way affect your legal rights.

5 What will happen to me if I take part?

A total of 84 individuals with dry mouth caused by radiotherapy will participate in this study. If you decided to take part, an appointment will be arranged at the study centre to see whether you fulfil the criteria for participation. Individuals who fulfil the criteria will participate into the study and will be divided into 2 groups of 42 each (group A and group B). Participants will be allocated into these 2 groups casually (a process known as randomisation) and neither participants nor investigators will know which patients belong to group A or B (known as "double blind design") until the end of the study. Participants of both groups will receive an individual customised device that is shaped on a cast (model) of their dentition taken with a routine dental impression (the same as that taken for dentures). Participants will be explained how to use the device and will be asked to bring it home and test it for 12 months. Participants of Group A (cases) will receive a device that releases electric stimuli while those of Group B (controls) consist of participants who will receive a device that does not release electric stimuli.

During this time, participants will be asked to attend 6 predefined hospital appointments (at month 1, 2, 4, 6, 8, 12; each appointment lasting about 30 minutes) in order to let doctors to assess the potential benefits of the device (doctors will measure salivation and will ask participants to complete 3 questionnaires – they will also check the device and examine the mouth of study participants). After measurement, saliva will be stored in freezer (in anonymised containers) and subsequently analysed for changes in its composition pre and posttreatment with electrostimulation.

After the study is completed, we may store remaining saliva samples in our Tissue Bank for possible use in future research upon review by ethical committee.

Participants will also be asked to report into a home diary the frequency of device application per day and degree of dryness per week.

6. Expenses and payments

We will reimburse your travel expenses and will compensate you for your time you will spend to attend hospital appointments relevant to the study.

7. What will I have to do?

You will be asked to bring the device home and use it as directed for the duration of the study (12 months). A dedicated remote control will also be provided to switch the device on/off.

We shall recommend keeping the device in its protective case to avoid damage (suck as breaks or heat deformation).

You will not be allowed to use pilocarpine tablets (a medication which is sometimes prescribed for dry mouth) during the study but you can continue to use your usual topical treatment (e.g. spray, mouthwash) for dry mouth control.

You will also be asked to attend pre-arranged hospital appointments for measurement and to fill questionnaires regarding the degree of dry mouth.

We will provide a home diary for you to report the frequency of device application per day and degree of dryness per week.

8. Alternatives

Treatment of dry mouth consists of:

(i) Salivary substitutes (mouthwash, spray, gel), which are applied into the mouth and typically provide only mild and transient benefit;

(ii) Tablets of pilocarpine, a medication that can stimulate your own salivation but is often burdened by adverse side effects.

Overall treatment modalities for dry mouth are unsatisfactory and many individuals attempt to lessen dry mouth sensation with frequent sips of water.

9. What are the possible disadvantages and risks of taking part?

Previous studies showed that the device is safe and its use does not cause discomfort as it is custom-made on the shape of individual dentition. Occasionally the device may cause friction and irritation to the lining of the mouth – this can be promptly resolved by the investigators (study doctors) who can easily re-shape the device and remove the irritating parts. The device may affect you speech but only while you are wearing it. As with any form of oral appliances or dental restorations (e.g. dentures, fillings and orthodontic treatment), it is possible that small broken fragments from the device may accidentally be ingested or inhaled. A case of device breakage, which went unnoticed and caused no harm, has been reported. We recommend informing the study Investigators right away if this occurs, and they will assess your condition.

10. What are the possible benefits of taking part?

Study participants will benefit from using a novel medication-free therapeutic means that – based on previous research - is likely to lessen their dry mouth sensation. Also participants in the control group (those using the non-functional device) should benefit from increase salivation due to tactile stimulation of the device onto the mouth – although this is expected to be lower than that caused by the electric stimuli.

11. What happens when the research study stops?

On study completion, participants will be required to return the device - as it is designed to operate for no longer than 13 months from the manufacturing date – and will be offered the return to their usual NHS clinics and receive currently available treatment means of dry mouth. Information will also be provided regarding the modality of purchasing a new device from the manufacturer (should they wish so).

It is anticipated that the results of the present study, if positive, will contribute towards device availability on NHS prescription – which means that cancer patients could receive it free of charge on the basis of their medical exemption (MedEx) certificate.

12. Will my taking part in the study be kept confidential?

The Investigator (study doctor) will make every possible effort to keep your personal information confidential. All the information collected will be kept by the research coordinator. The Chief Investigator is responsible for safety and security of the data. Medical records which identify you and the consent form signed by you may be inspected by an Institutional Review Board or Ethical Review Committee. The results of this research project may be presented at meetings or in publications; however, any research data released or published will not identify volunteers by name. All data and results will be completely anonymised and it will be impossible to identify you from them.

13. What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. Contact numbers of study investigators are provided at the end of this document – as an alternative, you can also complain directly to UCLH.

If you are harmed by taking part in this research project, there are no special compensation arrangements – although the normal NHS complaints mechanisms will still be available to you (where appropriate). If you are harmed due to someone's negligence, you may have legal grounds for compensation, but you may have to pay for it.

14. Study results

The results of this research study may be presented at meetings and may be published, likely at least one year after the end of the project. No patients will be identified in any report. If you would like to receive the results of the study, please contact the Chief Investigator, Prof. S. Porter, or Principal Investigator, Dr S Fedele by phone, letter or email.

15. Who is organising and funding the research

The study is sponsored by UCLH and funded by the National Institute for Health Research (NIHR)

16. Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Sheffield Research Ethics Committee

13.Whom to ask questions regarding this study or to make a complaint to

You have the right to ask questions concerning this study at any time - please contact **Prof. Stephen Porter** at 020 7916 1142 or **Dr Stefano Fedele at** 020 7916 1004.

Complaints

- To Study Investigators: please contact **Prof Stephen Porter** or **Dr Stefano Fedele**.
- To UCLH: please speak to the person in charge of the ward or clinic, or to our **Patient Advice and Liaison Service (PALS)** who will help with your problem quickly and informally. Contact PALS on 020 7380 9975.
- You can also make formal complaint. You can do this within 12 months of the events concerned, or within 12 months of becoming aware of the problem. Please write with full details to the **Complaints Manager** at: Governance Department, UCLH, 2nd Floor West, 250 Euston Road, London, NW1 2PG (Fax: 02073809595- email: complaints.officer@uclh.nhs.uk).

A copy of this information sheet and a signed consent form will be given to you.

Version 5, 28th November 2012
Study Number: OM-11-03
Patient Identification Number for this trial:

Title of project:

Study on a novel medical device for the treatment of reduced salivation (dry mouth) resulting from radiation therapy

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[Scientific title: **L**ong-term **E**valuation of the effectiveness **O**f a **N**ovel **I**ntra-oral electro-stimulator for the treatment of ra**D**iotherapy-**AS**sociated dry mouth (The LEONIDAS-2 study)].

Name of Chief Investigator: **Prof. Stephen Porter**
Name of Principal Investigator: **Dr. Stefano Fedele**

Please initial box

1. I confirm that I have read and understood the information sheet Version 6 dated 28th November 2012 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by the researchers and responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to my General Practitioner and General Dental Practitioner being informed of my participation in the study. ☐
5. I understand that the medical device to be investigated meets the requirements of all relevant European Directives (CE Marking) ☐
6. I understand that this study will investigate whether a novel medical device will lessen dry mouth sensation caused by radiotherapy to the Head and Neck area (provided to treat head and neck cancer). I also understand that saliva sample will be stored in freezers during the study and analyzed for changes in composition of saliva. ☐
7. I understand that the saliva sample taken from me may be stored after the study is completed and use for potential future research at a later date upon review by ethical committee. I understand that these results will remain anonymous. ☐
8. I agree to take part in the above study ☐

Name of patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

8.3 Home Diary

Patient Initials:_______	Study (Randomisation) n:_______

Long- term Evaluation of the effectiveness Of a Novel Intra-oral electro-stimulator for the treatment of raDiotherapy-ASsociated dry mouth (The LEONIDAS-2 study)
Study OM-11-03

Study start date (first day of use of the device): __/__/__

Month 1

Day	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Date	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Frequency (per day)							
Date	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Frequency (per day)							
Date	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Frequency (per day)							
Date	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Frequency (per day)							
Date	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Frequency (per day)							
Date	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Frequency (per day)							

End of Month 1: __/__/__

8.4 Quality of Life questionnaires

Table 8-1 OHQoL-16 questionnaire

What effect does your oral health (teeth, gums and mouth) have on your…		Very Bad	Bad	None	Good	Very Good
1	eating or enjoyment of food?					
2	appearance?					
3	speech?					
4	comfort (lack of pain and discomfort)?					
5	breath odor?					
6	general health?					
7	smiling or laughing?					
8	social life?					
9	romantic relationships?					
10	work or ability to do your usual jobs?					
11	finance?					
12	ability to relax or sleep?					
13	confidence (lack of embarrassment)?					
14	carefree manner (lack of worry)?					
15	mood or happiness?					
16	personality?					

Table 8-2 EORTC QLQ - H&N35 questionnaire

EORTC QLQ - H&N35

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week:		Not at all	A little	Quite a bit	Very much
31.	Have you had pain in your mouth?	1	2	3	4
32.	Have you had pain in your jaw?	1	2	3	4
33.	Have you had soreness in your mouth?	1	2	3	4
34.	Have you had a painful throat?	1	2	3	4
35.	Have you had problems swallowing liquids?	1	2	3	4
36.	Have you had problems swallowing pureed food?	1	2	3	4
37.	Have you had problems swallowing solid food?	1	2	3	4
38.	Have you choked when swallowing?	1	2	3	4
39.	Have you had problems with your teeth?	1	2	3	4
40.	Have you had problems opening your mouth wide?	1	2	3	4
41.	Have you had a dry mouth?	1	2	3	4
42.	Have you had sticky saliva?	1	2	3	4
43.	Have you had problems with your sense of smell?	1	2	3	4
44.	Have you had problems with your sense of taste?	1	2	3	4
45.	Have you coughed?	1	2	3	4
46.	Have you been hoarse?	1	2	3	4
47.	Have you felt ill?	1	2	3	4
48.	Has your appearance bothered you?	1	2	3	4

Please go on to the next page

During the past week:		Not at all	A little	Quite a bit	Very much
49.	Have you had trouble eating?	1	2	3	4
50.	Have you had trouble eating in front of your family?	1	2	3	4
51.	Have you had trouble eating in front of other people?	1	2	3	4
52.	Have you had trouble enjoying your meals?	1	2	3	4
53.	Have you had trouble talking to other people?	1	2	3	4
54.	Have you had trouble talking on the telephone?	1	2	3	4
55.	Have you had trouble having social contact with your family?	1	2	3	4
56.	Have you had trouble having social contact with friends?	1	2	3	4
57.	Have you had trouble going out in public?	1	2	3	4
58.	Have you had trouble having physical contact with family or friends?	1	2	3	4
59.	Have you felt less interest in sex?	1	2	3	4
60.	Have you felt less sexual enjoyment?	1	2	3	4

During the past week:		No	Yes
61.	Have you used pain-killers?	1	2
62.	Have you taken any nutritional supplements (excluding vitamins)?	1	2
63.	Have you used a feeding tube?	1	2
64.	Have you lost weight?	1	2
65.	Have you gained weight?	1	2

Table 8-3 SF-36 questionnaire

1	In general, would you say your health is: (Please tick **one** box) Excellent ☐ Very Good ☐ Good ☐ Fair ☐ Poor ☐			
2	Compared to one year ago, how would you rate your health in general now? (Please tick **one** box) Much better than one year ago ☐ Somewhat better now than one year ago ☐ About the same as one year ago ☐ Somewhat worse now than one year ago ☐ Much worse now than one year ago ☐			
3	The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? **(Please tick once on each statement)**			
	Activities	Yes, limited a lot	Yes, limited A little	Not limited at all
3(a)	**Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports			
3(b)	**Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf			
3(c)	Lifting or carrying groceries			
3(d)	Climbing **several** flights of stairs			
3(e)	Climbing one flight of stairs			
3(f)	Bending, kneeling, or stooping			
3(g)	Waling **more than a mile**			
3(h)	Walking **several blocks**			
3(i)	Walking **one block**			
3(j)	Bathing or dressing yourself			
4	During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? **(Please tick once on each statement)**		**Yes**	**No**
4(a)	Cut down on the amount of time you spent on work or other activities			
4(b)	Accomplished less than you would like			
4(c)	Were **limited** in the **kind** of work or other activities			
4(d)	Had **difficulty** performing the work or other activities (for example, it took extra effort)			
5	During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (e.g. feeling depressed or anxious)? **(Please tick once on each statement)**		**Yes**	**No**
5(a)	Cut down on the **amount of time** you spent on work or other activities			
5(b)	Accomplished less than you would like			
5(c)	Didn't do work or other activities as **carefully** as usual			

6 During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups? (Please tick **one** box)

- Not at all [ ]
- Slightly [ ]
- Moderately [ ]
- Quite a bit [ ]
- Extremely [ ]

7 How much physical pain have you had during the past 4 weeks? (Please tick **one** box)

- None [ ]
- Very mild [ ]
- Mild [ ]
- Moderate [ ]
- Severe [ ]
- Very Severe [ ]

8 During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)? (Please tick **one** box)

- Not at all [ ]
- A little bit [ ]
- Moderately [ ]
- Quite a bit [ ]
- Extremely [ ]

9 These questions are about how you feel and how things have been with you during the past 4 weeks. Please give the one answer that is closest to the way you have been feeling for each item.

(Please tick once on each statement)		**All of the time**	**Most of the time**	**A good bit of the time**	**Some of the time**	**A little of the time**	**None of the time**
9(a)	Did you feel full of life?						
9(b)	Have you been a very nervous person?						
9(c)	Have you felt so down in the dumps that nothing could cheer you up						
9(d)	Have you felt calm and peaceful?						
9(e)	Did you have a lot of energy?						
9(f)	Have you felt downhearted and blue?						
9(g)	Did you feel worn out?						
9(h)	Have you been a happy person?						
9(i)	Did you feel tired?						

10 During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives etc.) (Please tick **one** box)

- All of the time ☐
- Most of the time ☐
- Some of the time ☐
- A little of the time ☐
- None of the time ☐

11 How TRUE or FALSE is each of the following statements for you?

(Please tick once on each statement)		**Definitely True**	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
11(a)	I seem to get sick a little easier than other people					
11(b)	I am as healthy as anybody I know					
11(c)	I expect my health to get worse					
11(d)	My health is excellent					

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