Salivary gland sparing radiotherapy

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Voor mijn ouders
Voor Roos

Contents


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1 Introduction and outline

Head-and-neck cancer

In 2010, nearly 2900 patients received a diagnosis of head-and-neck cancer (HNC) in The Netherlands. This accounts for 3% of all newly diagnosed cancers annually and makes HNC a relatively rare malignancy. Roughly, two-third of the patients are male with incidence peaking between 60 and 74 years of age. Tumors of the pharynx and oral cavity account for almost 60% of all HNCs [1].

Radiotherapy (RT) and surgery are the mainstay of treatment for HNC. Either in de postoperative setting or as primary treatment, RT aims to eradicate tumor cells by causing (irreparable) damage to cellular DNA. Chemotherapy is often used together with RT in order to deal with the possible systemic spread of tumor cells (spatial cooperation) or because of its interaction with ionizing radiation at the cellular and molecular level [2].

Depending on the cancer staging and the pre-RT treatment, the primary tumor or postoperative tumorbed is irradiated together with any (suspected) lymph node metastases found in the neck during the diagnostic work-up. In selected cases where no macroscopic (visible) metastases are detected, but the risk of microscopic disease in the neck nodes is deemed high due to the tumor characteristics, elective radiation treatment is given to specific lymph node regions in the ipsi- and/ or contralateral neck. Taken altogether, radiotherapy for HNC often implies high radiation doses being delivered to relatively large target volumes. Due to the anatomical complexity of the head-and-neck region, these target volumes are situated in the vicinity of healthy organs such as the salivary glands, swallowing structures, spinal cord, vocal cords, skin and the mucosa of the oral cavity and pharynx. Particularly in (oro)pharyngeal tumors, all these organs are at risk for significant radiation damage.

Saliva production and xerostomia

Healthy individuals produce between 1 and 1.5 liter of saliva each day. Whole saliva is a complex mixture of secretions from the major and minor salivary glands and from gingival crevicular fluid, which contains bacteria and food debris [3].

The major salivary glands include the paired parotid glands, situated anterior to the auricle of the ear, and the paired submandibular and sublingual glands which are located in the floor of the mouth. The major glands produce 90% of the saliva volume. The remainder is produced by the minor glands, which are scattered throughout the oral cavity and pharynx. In a stimulated state such as during a meal, the parotid glands produce about 60-65% of the salivary volume and 20-30% is produced by the submandibular and sublingual glands. In a resting state this picture drastically changes, with the submandibular glands producing up to 90% of the total saliva output [4]. The quality of the different secretions also varies, depending on the type of acinar cells present in the glands. The parotid gland contains only serous acinar cells and its saliva consists almost entirely of water. Minor glands are made up of mucinous acini, which produce viscous saliva containing high concentrations of mucins. Submandibular and sublingual secretions are mixed serous and mucinous.

Saliva is critical for the maintenance of oral health and generally receives little attention until quantity or quality diminish. It has important buffering and antibacterial capacity, lubricates and protects oral tissues, prevents dental caries and facilitates speech. Saliva also enhances the taste sensation and it begins the digestive process of food [3]. Xerostomia includes the objective reduction of the salivary output and changes in its composition together with the subjective symptoms reported by the patient [5]. Radiation-induced damage to the salivary glands alters the volume, consistency and pH of secreted saliva. Patients suffer from oral dryness or pain; their saliva becomes thick, sticky and patients find it difficult to speak, chew or swallow. They are at risk for oral ulceration, infections and the accelerated decay of teeth. Ultimately, this can lead to decreased nutritional intake and weight loss, posing a major (secondary) health problem for patients [6].

The pathophysiology of salivary gland dysfunction after RT is somewhat enigmatic. Salivary glands belong to the group of acute responding tissues, despite the fact that the functional (acinar) cells are highly differentiated and almost non-cycling. Salivary flow decreases very early (within days) in the course of fractionated radiotherapy, while

no increased acinar cell loss is observed. Defects in water excretion, secondary to plasma membrane damage and disturbed signal transduction, are suggested to be the cause of the observed acute effect of radiation [7]. Late damage on the other hand, appears more classical in nature. It can be explained by a lack of progenitor and stem cells and radiation damage to the extracellular environment (salivary ducts, blood vessels). This causes a shortage of properly functioning secretory cells. Most research on (early) radiation damage has been performed in rats however, and the question remains if these findings can be extrapolated to humans.

Patient-reported xerostomia and quality of life

Assessing xerostomia is not a straightforward task. The severity of xerostomia is often underestimated by physicians, compared with that reported by patients. Moreover, observer-rated xerostomia is moderately reproducible and lacks correlation with salivary output. Patients self-reported, rather than physician-assessed scores, should be the main endpoint when evaluating xerostomia [8]. Several xerostomia-specific questionnaires have been developed and tested for their validity and reliability [9,10]. In general, they ask patients to rate difficulties related to xerostomia in chewing, swallowing, talking, during sleep and their need to drink water while eating dry food or while at rest. The different aspects are often summarized into a composite score. This should be done with caution however, because not all symptoms are specific to radiation-induced xerostomia. For example, swallowing problems can also be caused by damage to the pharyngeal constrictors after RT [11] and eating difficulties may arise secondary to mucositis early after irradiation.

Irrespective of xerostomia being measured by the physician or the patient, radiation-induced xerostomia is the most frequently reported adverse effect after RT for HNC and together with swallowing dysfunction has a highly significant impact on the quality of life of patients [12,13]. This impact tends to increase over time. Younger patients in particular frequently report disturbances in daily social and physical functioning secondary to xerostomia-related side effects [14].

Intensity-modulated radiotherapy (IMRT)

Efforts to reduce the severity of radiation-induced xerostomia include the use of salivary substitutes (artificial saliva), salivary gland stimulants (pilocarpine) or radioprotectants to be administered during the course of RT (for example amifostine). These interventions either failed to show clear efficacy with respect to patient-reported xerostomia in clinical studies or did show an unfavorable toxicity profile. At present, there is no clinically established treatment of xerostomia after RT [6,15]. Progress in the development of advanced radiation techniques has shifted the main focus of xerostomia research towards the prevention of salivary gland damage.

External beam irradiation has evolved over the past two decades from conventional techniques using large (opposing) radiation fields to three-dimensional (3D) conformal techniques with the possibility to more-or-less shape the dose around the tumor (target). IMRT is an advanced form of 3D conformal radiotherapy that uses non-uniform radiation beam intensities determined by computer-based optimization techniques [16]. It allows for concave and irregularly shaped dose distributions around target volumes and offers better sparing of organs at risk. The IMRT delivery system uses a multileaf collimator (MLC) to modulate beam intensity. A MLC consists of thin blades that can be individually positioned and can create irregular beam shapes. IMRT can be delivered either by superimposing a number of small static field shapes (segments) from different gantry angles (step-and-shoot delivery) or by a sliding window technique in which the gantry and MLC leaves move continuously during irradiation (dynamic arc delivery) [17].

IMRT has many advantages in HNC. (Irregular) target volumes and organs at risk in the head-and-neck region are very proximate, requiring sharp dose gradients to deliver (higher) therapeutic doses to the primary tumor while sparing healthy tissues like the salivary glands from toxicity [18]. See *Figure 1.* In order to achieve this, sophisticated strategies for patient immobilization and positioning, image-guided treatment planning and (computed tomography based) treatment verification are required.

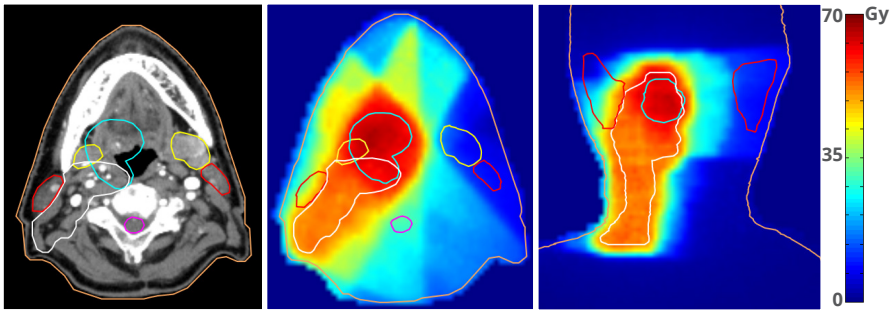

Figure 1 Sparing the salivary glands with IMRT in a patient with cancer of the right tonsillar region. On the left (a) the planning CT-scan is shown with target volumes and normal tissues delineated. The planning target volume (PTV) for the primary tumor is shown in blue, the white line represents the PTV for the elective lymph node area. The parotid glands are delineated in red, the submandibular glands in yellow. The purple line encompasses the spinal cord. On the right, in (b) and (c), the dose distributions obtained with IMRT can be seen in transversal and coronal directions, respectively. In particular, the left parotid and submandibular gland are spared from high radiation doses in this patient. Dose in Gray (Gy).

Quantifying salivary gland function after RT

Several methods exist to evaluate the effect of salivary gland sparing. Subjective methods like questionnaires were discussed previously. Alternatively, objective measures are applied to either directly measure salivary output or to image the functional activity of the salivary glands [6]. The parotid glands have been studied extensively in this way, because their sparing became feasible early after the clinical introduction of IMRT.

The total saliva production by all glands collectively (whole saliva; containing both major and minor gland saliva) can be measured by spitting, drooling or by weighing cotton rolls inserted into the mouth. Studies of dose-response relationships in the salivary glands are most reliable however, when selective measurements of gland function are performed. Selective collection of parotid gland saliva is done by applying a suction cup (Lashley cup) at the orifice of Stensen's duct in the buccal mucosa (*Figure 2*). The collection of submandibular and sublingual saliva can be performed by gentle suction with a micropipette near Wharton's duct orifices in the floor of the mouth. Unfortunately, there is no straightforward method available for selectively measuring minor salivary gland function [5].

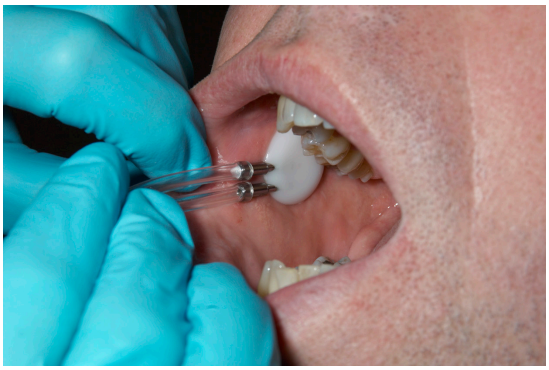

Figure 2 Selective collection of parotid gland saliva by applying a Lashley cup at the orifice of Stensen's duct.

Saliva can be measured unstimulated or stimulated, either by applying a stimulant like citric acid (2-5%) to the mobile part of the tongue or by a mechanical stimulus like chewing. The collected saliva is weighed and the volume is determined by assuming a specific gravity of 1.0 g/ ml, and the flow rate is reported in ml/ min. Saliva measurements may not be comparable between studies because of differences in the nature and length of application of the stimulus, differences in the collection methods and their duration, and the neglect of factors that can influence salivary output [5]. The latter include medications taken by the patient and diurnal variations in salivary flow. Standardizing saliva measurements is thus of great importance, even more so because the intraindividual variation of flow rates in healthy volunteers is reported to be as high as 27-44% (range) [19]. This also impedes the definition of a threshold of saliva output with which to define xerostomia. Arbitrarily, when modelling dose-response relationships of the salivary glands, a reduction of salivary flow to ≤25% of the pre-RT flow rate is considered relevant (Grade 4 xerostomia according to the Radiation Therapy Oncology Group/ European Organization for Research and Treatment of Cancer (RTOG/ EORTC) Late Effects Consensus Conference) [20]. For parotid gland function after RT, it was shown that stimulated (Lashley cup) measurements using this threshold correlated best with the parotid gland mean dose [21].

Imaging the functional activity of the salivary glands can be performed by scintigraphy with ^{99M}Tc-pertechnetate. It can detect the glands'

ability to collect and excrete saliva to small amounts [22]. Scintigraphy is expensive however, more invasive and requires dedicated nuclear medicine expertise. Although parotid gland scintigraphy also showed a significant correlation with mean dose after RT, a direct comparison identified stimulated flow measurements as the preferred method for evaluating parotid gland function after RT [21].

Qualitative aspects of saliva

The correlation between residual (parotid) salivary flow and patient-reported xerostomia scores is generally poor. This may be caused by the variation in salivary flow rates and by discrepancies between the measured salivary output and the hydration status of the mucosa [5]. Mucins play an important role in this respect. They are high-molecular weight glycoproteins able to retain large amounts of water and contribute to the formation of a thin salivary film which is thought to hydrate and lubricate the soft tissues of the mouth [23]. Mucins are produced by mucinous acinar cells found in the submandibular, sublingual and in particular the minor salivary glands. Serous secretions from the parotid glands lack mucins.

The mean radiation dose to the submandibular glands and oral cavity were previously identified as independent and statistically significant factors predicting the severity of patient-reported xerostomia [9,24]. The oral cavity mean dose can be viewed as a surrogate for radiation damage to the minor salivary glands. These findings provide indirect support for the hypothesis that mucin-rich saliva from the submandibular and minor salivary glands plays an important role in the perception of dry mouth.

From a clinical point-of-view, sparing the (non-involved) oral cavity with IMRT is very challenging due to the close proximity of the primary tumor, especially in cancers of the oropharynx and oral cavity itself. Sparing of the (contralateral) submandibular gland may be a more feasible and realistic goal in most HNCs, in addition to sparing both parotid glands.

Outline of this thesis

The research described in this thesis addresses the sparing of (major) salivary gland function after RT for head-and-neck cancer. This requires detailed knowledge of the dose-response relationships for the major salivary glands, the influence of quantitative and qualitative saliva parameters on subjective symptoms and (pre)clinical data on the feasibility and efficacy of salivary gland sparing RT on patient-reported xerostomia.

Normal tissue complication probability (NTCP) models are used to describe the sigmoid relationship between radiation dose to an organ at risk and the chance of a significant decline in function (or complication) after RT. For the parotid gland, using selective flow measurements and mean dose as input parameters, two different NTCP-curves have been described in literature based on conventional and intensity-modulated RT. Different RT techniques can lead to differences in the dose distribution within the parotid gland, despite the same mean dose delivered. Chapter 2 examines the descriptive ability of the mean parotid gland dose on NTCP up to 1 year after RT.

Based on the findings in chapter 2, we constructed a definite NTCP-curve for parotid gland function at 1 year after RT by combining dose-response data from the University Medical Center Utrecht with data from the University of Michigan (chapter 3). This combination of multi-institutional experience provides the largest dataset of parotid gland function after RT published to date.

Chapter 4 examines the differential impact of post-RT parotid and submandibular gland function on patient-reported xerostomia. It was hypothesized that the quantitative and qualitative differences in saliva output from the major salivary glands could have a different impact on the feeling of dry mouth during daytime and at night (literally a 'resting state'). Extending this hypothesis with the observation that gains in patient-reported xerostomia after parotid gland sparing IMRT alone have been relatively small, we performed a pilot study to investigate if MUC5B levels in submandibular gland saliva could better distinguish between the presence or absence of severe dry mouth complaints at 1

year after RT for HNC (chapter 5). MUC5B is the largest mucin present in (submandibular gland) saliva and is thought to be related to the perception of dry mouth by keeping the oral mucosa in a hydrated state.

To date, relatively sparse data exist concerning the dose-response relationships for the submandibular glands after RT. To aid IMRT treatment planning, these relationships are described in chapter 6. Using data from a large cohort of HNC patients, NTCP-modelling of submandibular gland function at 6 weeks and 1 year after RT was performed with mean dose and selective flow measurements as input parameters.

From an oncological point-of-view, sparing the submandibular glands with IMRT for HNC is challenging due to its location adjacent to the level II(a) lymph node region in the neck. Particularly in oropharyngeal cancer, lymph nodes in this region are at risk for (microscopic) metastases and are usually encompassed in the elective nodal clinical target volume (CTV). Moreover, the submandibular gland ipsilateral to the primary tumor receives high radiation doses in most patients. The contralateral submandibular gland mean dose can potentially be reduced, but challenges remain due to its anatomical relationship with the elective nodal CTV. Chapter 7 describes the development of an advanced IMRT-technique for sparing the contralateral submandibular gland, in addition to both parotid glands, in oropharyngeal cancer patients without contralateral lymph node metastases.

Chapter 8 presents the initial results from a prospective cohort study investigating the effect of sparing the contralateral submandibular gland and both parotid glands on patient-reported xerostomia in oropharyngeal cancer. Using advanced IMRT, we aimed to spare the contralateral submandibular gland mean dose below 40 Gy in patients without (evidence of) contralateral lymph node metastases. This new cohort was compared with a historical cohort of oropharyngeal cancer patients that had received only parotid gland sparing IMRT.

Finally, chapter 9 summarizes the most important results presented in this thesis and discusses the impact of salivary gland sparing radiotherapy on patient-reported xerostomia in HNC.

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2 Large cohort dose-volume response analysis of parotid gland function after radiotherapy: intensity-modulated versus conventional radiotherapy

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Abstract

Purpose: To compare dose-volume response relationships in a large cohort of patients treated with intensity-modulated (IMRT) and conventional radiotherapy (CRT).

Patients and methods: 221 patients (64 treated with IMRT, 157 with CRT) with various head-and-neck malignancies were prospectively evaluated. The distribution of tumor subsites in both groups was unbalanced. Stimulated parotid flow rates were measured before and 6 weeks, 6 months and one year after radiotherapy. Parotid gland dose-volume histograms were derived from CT-based treatment planning. The normal tissue complication probability (NTCP) model proposed by Lyman was fit to the data. A complication was defined as stimulated parotid flow ratio <25% of the pre-treatment flow rate. The relative risk of complications was determined for IMRT versus CRT and adjusted for the mean parotid gland dose using Poisson regression modelling.

Results: At 1 year after radiotherapy, NTCP curves for IMRT and CRT were comparable with a TD_{50} (uniform dose leading to a 50% complication probability) of 38 and 40 Gy, respectively. Until 6 months after RT, corrected for mean dose, different complication probabilities existed for IMRT versus CRT. The relative risk of a complication for IMRT versus CRT after 6 weeks was 1.42 (95% CI; 1.21-1.67), after 6 months 1.41 (95% CI; 1.12-1.77) and at one year 1.21 (95% CI 0.87-1.68), after correcting for mean dose.

Conclusions: At 1 year after radiotherapy, no difference existed in the mean dose based NTCP curves for IMRT and CRT. Early after radiotherapy (up to 6 months) mean dose based models failed to fully describe the effects of radiotherapy on the parotid glands.

Introduction

Reduced salivary output (xerostomia) is the most common late side effects of radiotherapy (RT) for head-and-neck malignancies and a major cause of decreased quality of life in survivors [1]. Five years after conventional radiotherapy (CRT), approximately 40% of patients complain of moderate or severe xerostomia [2]. Intensity-modulated radiotherapy (IMRT) has the potential to reduce the dose to healthy tissue without compromising the dose to the tumor volume. We recently showed that IMRT significantly reduces the mean dose to the parotid glands and the number of parotid flow complications for patients with oropharyngeal cancer [3].

Dose-response relationships for the parotid gland based on dose-volume histograms have been determined using a variety of methods. These include whole-mouth [4,5] and selective parotid salivary flow rates [6-9], or functional imaging of the parotid glands using scintigraphy [10-12]. The common finding in all these studies is the strong correlation of the post-RT parotid gland function with the mean dose. Of the different methods used, stimulated parotid flow measurements using Lashley cups correlate best with mean parotid gland dose [13]. In most studies however, the data originates from relatively small patient groups. Mean dose may therefore not be the optimal descriptor of parotid flow after RT.

Several investigators have reported on the mean dose and residual parotid gland function after RT using objective flow measurements [5-8]. Normal tissue complication probability (NTCP) curves have been constructed, describing a sigmoid relationship between radiation dose, volume and probability of radiation-induced changes in parotid gland function. Two NTCP curves from relatively large patient groups have been published, both with mean dose as descriptor. Using the same method of parotid salivary flow measurement (Lashley cups), at one year, the tolerance dose for 50% complication probability for uniformly irradiated parotid gland (TD_{50}) ranges from 28 to 39 Gy. The slope of the dose-response curve also differs. A threshold-shaped curve (mean dose ≤26 Gy) was described by Eisbruch *et al.* [6], in a group

of 88 patients treated with an IMRT-technique. Our group found no threshold dose in a study of 108 patients with various head-and-neck malignancies treated with CRT [7]. These differences possibly arise from the fact that different RT techniques were used, which may cause different relationships between the mean dose and partial volumes receiving any specified dose [3,14-16]. Different techniques may spare different parts of the parotid gland, leading to different function post-RT for the same mean dose.

The aim of this study was to prospectively investigate, using a large cohort of patients, the relationship between the mean dose to the parotid gland and complication probability for CRT and IMRT. Thereby, we examined the descriptive ability of the mean parotid gland dose in a clinical setting.

Patients and methods

Patients

From 1996 to 2007, a total of 221 patients with histologically proven carcinoma were prospectively evaluated in salivary gland function studies at our department. Of these, 157 were treated with CRT and 64 with IMRT. We have reported previously on the first 108 conventionally treated patients with various head-and-neck malignancies [7,17]. Recovery of parotid gland function was shown at 6 months, one year [7] and five years [17] after RT. Another 45 patients participated in a double-blind, placebo-controlled, randomized clinical trial investigating the effect of pilocarpine on radiation-induced xerostomia [18]. Only the patients who received a placebo in that trial were included in the analysis presented here. Four additional patients received postoperative 3D radiation treatment. All these patients were combined together in the conventional radiotherapy (CRT) group. The IMRT group consisted of 64 patients with mainly oropharyngeal and nasopharyngeal tumors included in an ongoing prospective study on parotid gland sparing radiotherapy at our department. These patients were selected for study because most parotid gland sparing can theoretically be achieved in patients with oropharyngeal and nasopharyngeal cancer.

None of the patients received previous radiotherapy to or surgery of the parotid glands or had other diseases of the parotid glands. Patients with evidence of distant metastatic disease were not included in the study, and a World Health Organization status of 0 or 1 was required. Fourteen patients in the IMRT group received concomitant cisplatinum-based chemotherapy (100 mg/ m^2) on day 1, 22 and 43 after the start of radiotherapy. The main indication for concomitant chemotherapy was bulky tumor disease or invasion of the base of the skull in case of nasopharyngeal cancer. Further use of any medication known to affect salivary gland function was prohibited.

All studies described above were approved by the Medical Ethical Committee of the University Medical Center Utrecht. Informed consent was obtained from each patient.

CRT

Details on radiation treatment planning have been reported previously [3,7]. A total of 157 patients received external beam radiotherapy with 6 MV photons using isocentric techniques. In the majority of patients, opposing lateral fields were used for target volume coverage and an anterior field was used for the supraclavicular regions. Electron beams were used to boost the posterior neck region after shielding the spinal cord at 40-46 Gy. The radiation dose varied with diagnosis, according to generally accepted treatment strategies. Four additional patients were treated using 3D radiation treatment planning (PLATO RTS 2.0, Nucletron BV, Veenendaal, The Netherlands).

For each patient, contrast-enhanced CT imaging of the head-and-neck region including the major salivary glands was performed with 3 mm thick slices. When treatment fields were designed using radiographs, reconstruction took place on the CT slices using the patient's setup marks.

Daily fractions of 2 Gy were given 5 days per week. Thirteen patients with advanced laryngeal cancer participated in an institutional protocol and received hyperfractionated accelerated radiotherapy [19]. Prescribed target doses were 46-50 Gy for the clinically negative, undissected neck at risk for microscopic metastatic disease; 50-70 Gy for postoperative tumor beds or dissected neck sites, depending on

the results of the pathologic review of the operation specimen; and 66-70 Gy for tumors primarily treated with radiation. On average, patients were treated with 35 fractions (mean; range 20-50) delivered in 44 days (mean, range 26-65).

IMRT

Details on treatment planning and target delineation were reported recently [3,20]. Sixty-four patients received parotid-sparing, inverse-planned, step-and-shoot IMRT. Contrast-enhanced CT imaging with 3mm slice thickness was performed and matched with magnetic resonance imaging (MRI) and positron-emission tomography (PET) data, when available. The gross tumor volume (GTV), the clinical target volume (CTV) of the elective lymph nodes and organs at risk (spinal cord, brain, parotid glands) were delineated on each slide. The level II to IV neck nodes were included in the elective CTV and treated according to institutional guidelines. In case of nasopharyngeal cancer, level Ib and V were also included. IMRT plans were obtained using the inverse treatment-planning module PLATO-ITP, version 1.1 (Nucletron BV). Five equidistant beams were used, starting at 0°. After 21 patients had been treated, a seven-beam technique was applied. All plans were dosimetrically verified on the treatment machine using ionization chamber and film measurements. Verification of patient position was performed the first 3 fractions and then once a week. Patients were treated with daily fractions, 5 days per week.

Oropharyngeal cancer patients treated without chemotherapy received an integrated boost. For these patients, the prescribed dose to the GTV of the macroscopic tumor was 69 Gy in 2.3 Gy daily fractions and to the CTV of the GTV 66 Gy in 2.2 Gy daily fractions. For the elective irradiation of the lymph nodes a dose of 54 Gy in 1.8 Gy daily fractions was prescribed. Nasopharyngeal cancer patients and the patients with an oropharyngeal tumor receiving concomitant chemotherapy, were treated without integrated boost. In these patients, the primary tumor and the elective lymph nodes received 46 Gy in 2 Gy fractions, followed by a separate boost of 24 Gy in 2 Gy fractions to the primary tumor and positive lymph nodes, if present. Depending on the type of boost,

patients were treated with either 30 (integrated boost) or 35 (separate boost) fractions (mean 32 fractions) delivered in 44 days (mean, range 40-56).

Seven patients with nasopharyngeal cancer (T1-T2, N0-N1; TNM staging system, 2002) received a brachytherapy boost to the primary tumor two weeks after the course of external beam radiotherapy. Using a nasopharyngeal applicator, the tumor received a boost dose of 12 Gy in 3 Gy fractions twice daily (high dose rate at 6 hour interval).

In these patients, a partial volume of the parotid glands received a maximum of 10% (1.2 Gy) of the prescribed boost dose. This small additional dose was neglected in the analysis.

Parotid gland delineation

For each patient, the left and the right parotid glands were delineated on multiple axial CT slices. No differentiation was made between the deep and superficial lobes. Three-dimensional dose distributions in the glands were calculated using the 3D pencil beam convolution algorithm in the PLATO RTS planning system. Separate dose-volume histograms (DVHs) were generated for the right and the left parotid gland. Each gland was thus analyzed separately [3,7,17].

Parotid flow measurements

The parotid flow rates were measured before treatment and 6 weeks, 6 months and one year after radiotherapy, as described previously [3,7]. Bilateral stimulated parotid saliva was collected simultaneously from both parotid glands using Lashley cups, which were placed over the orifice of Stensen's duct. Stimulation was achieved by application of a 5% citric acid solution on the mobile part of the tongue. Patients were instructed not to eat or drink 60 min before saliva collection. The parotid flow rate measurements at each visit were converted into the percentage of baseline flow rates. A complication was defined for each individual gland as a stimulated parotid flow rate <25% of the pre-treatment flow rate, according to the RTOG/ EORTC Late Effects Consensus Conference [21].

Normal tissue complication probability model

The flow data were fit to the normal tissue complication probability (NTCP) model proposed by Lyman [22,23]. This model has been used previously to determine dose-response relationships in the parotid gland [6-7,9,17]. It is assumed to quantitatively establish the effects of both radiation dose and volume of the gland irradiated on the probability of radiation-induced changes in parotid gland function. The model requires input of a single parotid gland dose and therefore, the non-uniform (multistep) dose distribution is reduced to a single-step DVH with an effective volume irradiated uniformly by a reference dose (effective dose). The transformed histogram is assumed to have the same complication probability as the original histogram. Three parameters; TD_{50}, n and m are estimated from the sigmoid dose-response relationship in the Lyman equation. Parameter m describes the slope of the NTCP-curve; TD_{50} represents the uniform dose to the whole organ resulting in 50% complication probability.

Parameter n accounts for the volume effect of an organ and is used in the histogram reduction. It is high (close to or higher than 1) if partial sparing of the organ reduces the complication probability (parallel functional subunit architecture; as in liver, lungs, parotid glands) and close to 0 if partial irradiation induces dysfunction (serial architecture, as in spinal cord). We chose to fix n at 1 and thereby to represent the effective parotid gland dose by the mean dose. Parotid gland NTCP curves published to date have used the mean dose (n = 1) as descriptor [5-7,9]. To examine whether the mean dose describes the same complication probability in patients treated with IMRT versus CRT, we also used n = 1 in this study. Analysis of n values other than 1 is outside the scope of this analysis.

Statistical analysis

Patient characteristics and mean dose values were characterized using descriptive statistics (mean, median, standard deviation (SD), ranges or proportions; where appropriate). Statistical differences in proportions were tested using Chi-square test or Fisher's Exact test, where appropriate. Differences in continuous variables were tested with Student's *t*-test when normally distributed and with the Mann-Whitney *U* test otherwise.

The parotid salivary flow measurements were analyzed separately for the left and right parotid gland. The NTCP parameters *m* and TD_{50} were determined by a maximum likelihood estimation technique [7,9]. They were estimated separately for the CRT- and IMRT-group at each time interval after RT (6 weeks, 6 months and 1 year).

To explore the whole data range in more detail, the effect of radiation technique (IMRT versus CRT) on the probability of complications 6 weeks, 6 months and one year after RT was estimated by computing relative risks (RR) and accompanying 95% confidence intervals (CI). Modified Poisson regression models were used to adjust these relative risks for potential confounders [24]. The confounding effect of the following variables was studied one by one: age, gender, mean dose to the parotid gland (Gy), surgery (definitive RT versus postoperative RT) and number of days the patient was treated. Variables that changed the crude relative risks by more than 10% were included in the final model.

The IMRT-group, in contrast to the patients treated with CRT, included patients who also received chemotherapy or brachytherapy. The CRT-group included thirteen patients that were treated using a hyper-fractionated-accelerated schedule. Analyses were repeated excluding patients with chemotherapy, brachytherapy or altered fractionation to investigate how this affected the results.

All analyses were performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA) except for the modified Poisson regression analyses that were performed using the PROC GENMOD procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The two-tailed significance level was set at 0.05.

Results

Patient, tumor and treatment characteristics of the 221 patients are outlined in *Table 1*. In the CRT-group, proportionally more patients were male ($p = 0.011$). Tumor sites in both groups varied as a consequence of different inclusion criteria for the studies described in the Patients and Methods section. In the IMRT-group significantly more patients received definitive radiotherapy ($p = 0.0001$) and a small group received

Table 1 Patient, tumor and treatment characteristics, n (%).

	CRT *(n = 157)*	**IMRT** *(n = 64)*
Gender		
Male	120 (76)	38 (59)
Female	37 (24)	26 (41)
Age (y)		
Median	58	60
Range	24-99	43-88
Tumor site		
Larynx	72 (45)	-
Hypopharynx	4 (3)	2 (3)
Oropharynx	28 (18)	48 (75)
Nasopharynx	4 (3)	13 (20)
Oral cavity	25 (16)	-
Nasal cavity	8 (5)	-
Unknown primary	2 (1)	1 (2)
Other	14 (9)	-
Stage (TNM)		
T-stage		
T1-2	86 (54)	44 (69)
T3-4	45 (29)	19 (29)
Tx	3 (2)	1 (2)
NA/ recurrent	23 (15)	-
N-stage		
N0	87 (55)	22 (34)
Ipsilateral	46 (29)	29 (45)
Bi-/ contralateral	2 (1)	11 (17)
N3	-	1 (2)
NA/ recurrent	22 (14)	1 (2)
Radiotherapy		
Definitive	84 (53)	57 (89)
Postoperative		
Primary tumor	23 (15)	-
Neck dissection	4 (3)	6 (9)
Both	46 (29)	1 (2)
Chemotherapy	0 (0)	14 (22)
Brachytherapy boost	0 (0)	7 (11)

Abbreviations: CRT = conventional radiotherapy; IMRT = intensity-modulated RT; NA = not applicable.

adjuvant chemotherapy or brachytherapy, compared with none in the CRT-group.

The mean dose to the parotid gland was comparable for the CRT-group (34.7 Gy; SD 17.9) and IMRT-group (35.0 Gy; SD 10.9). The mean volume of the parotid gland was 26 cm^3 in the CRT group (range 5-51 cm^3; SD 8.2) and 26 cm^3 in IMRT (range 9-61 cm^3; SD 9.6). A total of 115 patients had three flow measurements performed taken after radiotherapy. The remaining patients had missed appointments, (recurrent) illness or were lost to follow-up. One female patient interrupted radiation for 6 weeks because of aspiration pneumonia and was admitted to the Intensive Care Unit. This patient was excluded from the analyses. Mean dose, crude complication rates and number of glands analyzed in the CRT- and IMRT-cohort are shown in *Table 2*.

Table 2 Parotid gland function parameters for patients treated with conventional radiotherapy (CRT) and intensity-modulated radiotherapy (IMRT).

Parameter	CRT	IMRT	*p*-value
Mean parotid gland dose (Gy)			
Mean	34.7	35.0	0.34
Range	0-68.7	3.1-64.7	
Flow complications			
6 weeks			
Number of glands	237	98	
Complications (%)	131 (55)	69 (70)	0.01
6 months			
Number of glands	189	74	
Complications (%)	87 (46)	44 (60)	0.05
1 year			
Number of glands	174	61	
Complications (%)	66 (38)	24 (39)	0.85

Figure 1 shows the parotid flow ratio at 6 weeks, 6 months and one year post-RT as a function of the mean parotid gland dose. The parotid flow ratio decreased with increasing mean dose at all timepoints post-RT. There were few data in the lowest dose bin of the IMRT-group at 6 months post-RT, probably influencing the slope of the NTCP curve at that timepoint. We chose to represent the crude data however, and did not add artificial zero-dose DVHs to the data set [9].

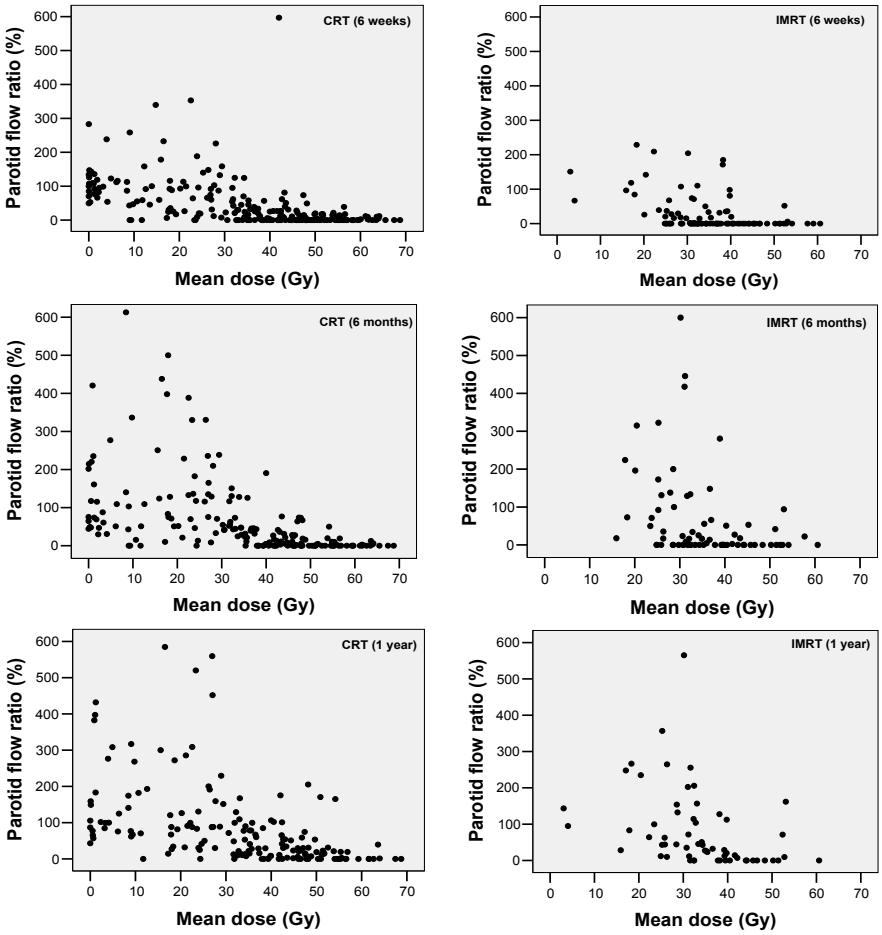

Figure 1. Parotid gland flow ratios as a function of the mean dose to the parotid gland at 6 weeks, 6 months and 1 year post-radiotherapy. Flow rates are expressed as the percentage of the pre-radiotherapy flow rates for each parotid gland. Flow ratios over 100% were observed as a result of the variability in (baseline) flow rates.
Abbreviations: CRT = conventional radiotherapy; IMRT = intensity-modulated RT.

The Lyman model was fit to the parotid gland flow data to determine NTCP-curves. *Table 3* gives the estimates for the parameters TD_{50} and *m* for the data at 6 weeks, 6 months and one year post-radiotherapy. A large difference between the TD_{50} for CRT- and IMRT-irradiated parotid glands existed at 6 weeks. At one year, this difference was no longer present. The clinical data and corresponding NTCP curves are depicted in *Figure 2*. No distinct threshold dose was found. Clear differences

were present for IMRT versus CRT for the mean dose range 20-40 Gy at 6 weeks and 6 months post-RT (p = 0.016 and p = 0.003, respectively). There was a tendency toward parotid gland function recovery over time for both groups.

Table 3 Lyman-parameters TD_{50} (Gy) and m estimated from the dose distribution and parotid flow data at 6 weeks, 6 months and 1 year post-radiotherapy.

Timing post-RT	CRT (95% CI)	IMRT (95% CI)
6 weeks		
TD_{50}	32 (29-34)	26 (22-29)
m	0.51 (0.42-0.64)	0.59 (0.46-0.79)
6 months		
TD_{50}	36 (33-39)	31 (26-35)
m	0.47 (0.38-0.59)	0.63 (0.61-0.85)
1 year		
TD_{50}	40 (37-44)	38 (35-42)
m	0.46 (0.37-0.59)	-0.33 (0.23-0.49)

Abbreviations: CI = confidence interval; TD_{50} = the uniform dose to the whole organ resulting in 50% complication probability; m = slope of the complication probability curve.

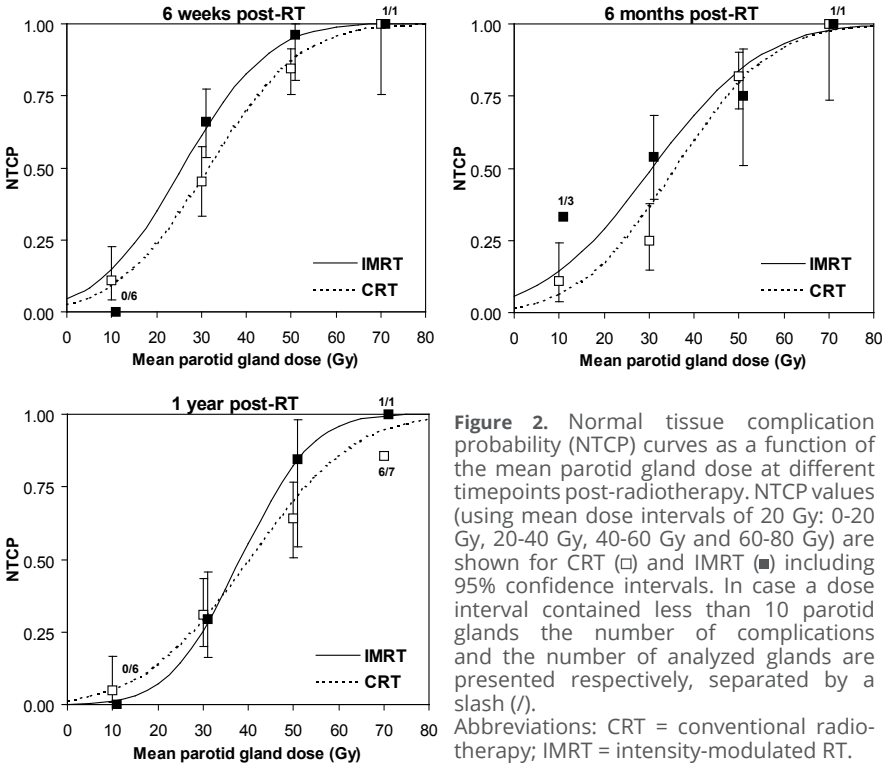

Figure 2. Normal tissue complication probability (NTCP) curves as a function of the mean parotid gland dose at different timepoints post-radiotherapy. NTCP values (using mean dose intervals of 20 Gy: 0-20 Gy, 20-40 Gy, 40-60 Gy and 60-80 Gy) are shown for CRT (□) and IMRT (■) including 95% confidence intervals. In case a dose interval contained less than 10 parotid glands the number of complications and the number of analyzed glands are presented respectively, separated by a slash (/).
Abbreviations: CRT = conventional radiotherapy; IMRT = intensity-modulated RT.

The crude relative risks of IMRT versus CRT for a parotid flow complication at the different timepoints post-RT are shown in *Table 4*. As expected, mean parotid gland dose strongly affected this relationship and was included in the model. For every Gy in mean dose, irrespective of treatment technique, the probability of a flow complication in the parotid glands increased 1.05 fold (one year after RT). No other confounding variables were identified. After adjusting for the mean dose, the RR of a flow complication in parotid glands of IMRT- versus CRT-treated patients at 6 weeks and 6 months was 1.42 (95% CI; 1.21-1.67) and 1.41 (95% CI; 1.12-1.77), respectively. In other words, adjusted for mean dose, the probability of a parotid gland complication after 6 weeks and 6 months is 1.4 times higher when IMRT is applied compared to CRT. One year post-RT, after correcting for mean dose, no effect existed of radiation technique on complication probability in the parotid gland (RR 1.21; 95% CI 0.87-1.68).

Table 4 Modified Poisson regression modeling of the effect of radiation technique (IMRT versus CRT) and mean parotid gland dose on complication probability in the parotid gland. Separate analyses were performed at 6 weeks, 6 months and 1 year post-radiotherapy.

Timing post-RT	Crude RR (95% CI)	Adjusted RR (95% CI) *	*p*-value †
6 weeks			
IMRT vs CRT	1.27 (1.07-1.51)	1.42 (1.21-1.67)	<0.0001
Mean dose (Gy)		1.04 (1.03-1.04)	<0.0001
6 months			
IMRT vs CRT	1.29 (1.01-1.65)	1.41 (1.12-1.77)	0.004
Mean dose (Gy)		1.04 (1.03-1.05)	<0.0001
1 year			
IMRT vs CRT	1.04 (0.72-1.49)	1.21 (0.87-1.68)	0.26
Mean dose (Gy)		1.05 (1.04-1.06)	<0.0001

Abbreviations: IMRT = intensity-modulated radiotherapy; CRT = conventional RT; 95% CI = 95% confidence interval; RR = relative risk.
* Adjusted relative risk (RR) of treatment technique (IMRT vs CRT) and mean parotid gland dose (in Gy) on the endpoint: parotid flow to <25% of baseline. The adjusted RR for 'IMRT vs CRT' is corrected for 'mean dose' and vice versa.
† *p*-value for the adjusted RR.

A separate analysis restricted to the patients that had flow rates measured at all 3 timepoints after RT (IMRT: 49 glands, CRT: 136 glands) yielded comparable significant results as in the original analysis: at 6 weeks $p = 0.003$; at 6 months $p = 0.002$ and one year after RT $p = 0.12$.

Repeating the analyses without the patients that received chemotherapy, brachytherapy or hyperfractionation did not materially change our results. The resulting adjusted RR at 6 weeks for IMRT versus CRT was 1.30 (95% CI 1.07-1.59; 69 versus 208 glands analyzed, $p = 0.01$). At 6 months, the adjusted RR was 1.44 (95% CI 1.11-1.87; 50 versus 165 glands analyzed, $p = 0.007$) and at one year 1.20 (95% CI 0.81-1.77; 46 versus 153 glands analyzed, $p = 0.12$).

Discussion

This is the first study comparing dose-response relationships of the parotid gland in patients treated with conventional (CRT) and intensity-modulated radiotherapy (IMRT) techniques, using objective saliva measurements. One year after the completion of radiation treatment, no difference existed in the complication (NTCP) curve of parotid glands in CRT- and IMRT-treated patients (*Figure 2*). Up to 6 months after RT, however, the mean dose did not fully describe early radiation-induced parotid gland damage. After correcting for the mean dose, we found that 6 weeks and 6 months after radiotherapy, parotid glands in IMRT-treated patients exhibit a higher risk of flow reduction to <25% of the pre-treatment flow rate.

The mean dose to the parotid gland and the percentage flow complications were higher for IMRT versus CRT (*Table 2*). This contradicts previous findings by us and by others. However, the distribution of tumor sites differed largely in both groups (*Table 1*), with laryngeal tumors only present in the CRT-group and predominantly oropharyngeal and nasopharyngeal tumors in the IMRT-group. Because the parotid glands are anatomically close to the oropharynx and nasopharynx, they generally receive a higher (mean) dose compared to patients with a tumor located in the larynx or elsewhere. For that reason, both groups could only be compared after correcting for mean parotid gland dose at all timepoints after RT. For oropharyngeal and nasopharyngeal cancer sec, it has already been proven that IMRT significantly reduces the mean dose and the number of parotid flow complications compared with CRT [3,25]. The main goal of this study was to examine the complication probabilities in IMRT- and CRT-treated patients after correcting for the mean parotid gland dose.

The observed differences in NTCP early post-RT are probably caused by the different spatial dose distribution obtained with CRT and IMRT. In both techniques, a different relationship exists between the mean dose and partial volumes receiving any specified dose. This is illustrated in *Figure 3* for the oropharyngeal cancer patients included in this study. The DVHs resulting from IMRT tend to be less steep than in conventional RT. Given the larger standard deviation for the mean parotid gland dose in the CRT group, the interpatient variability in the DVHs of all the CRT-treated patients will be larger than that for the IMRT-group.

In contrast to CRT, IMRT can create relative sharp dose-gradients in medial-lateral direction. This results in a relative high dose to the medial structures of the parotid gland (near the tumor) and a low-dose area in the peripheral gland tissue. With the CRT-techniques used, dose gradients generally exist in cranial-caudal direction only. Furthermore, the use of five to seven equidistant beams in IMRT adds a low dose field to the surrounding non-tumor tissue. We hypothesize that with IMRT, this low-dose area could cause significant damage to the excreting properties of the parotid gland early after radiation. The mechanism for this may be related to plasma membrane damage of acinar cells, which is suggested as the mechanism for early radiation damage in rodent (rat) parotid glands. The membrane damage, compromising these cells with respect to receptor-mediated water excretion, is enhanced early after radiation with low dose rates in rats [26]. On the other hand, a relatively high dose to the medial part of the parotid gland with IMRT could lead to more damage to the supplying structures such as ducts and blood vessels [27]. Both hypotheses are theoretical and have to be tested in detailed clinical delineation studies. This is not the aim of this study. Taken altogether, the mean dose (Lyman) model is not the optimal descriptor of early radiation effects in the parotid gland.

Late after RT (at one year), the mean dose based NTCP-curves were comparable with a TD_{50} of 38 Gy for IMRT versus 40 Gy for CRT. This suggests a potential for recovery in the IMRT-irradiated gland tissue to a level comparable with that after CRT. The renewal capacity of progenitor and stem cells, a marker of late injury, seems to be equally

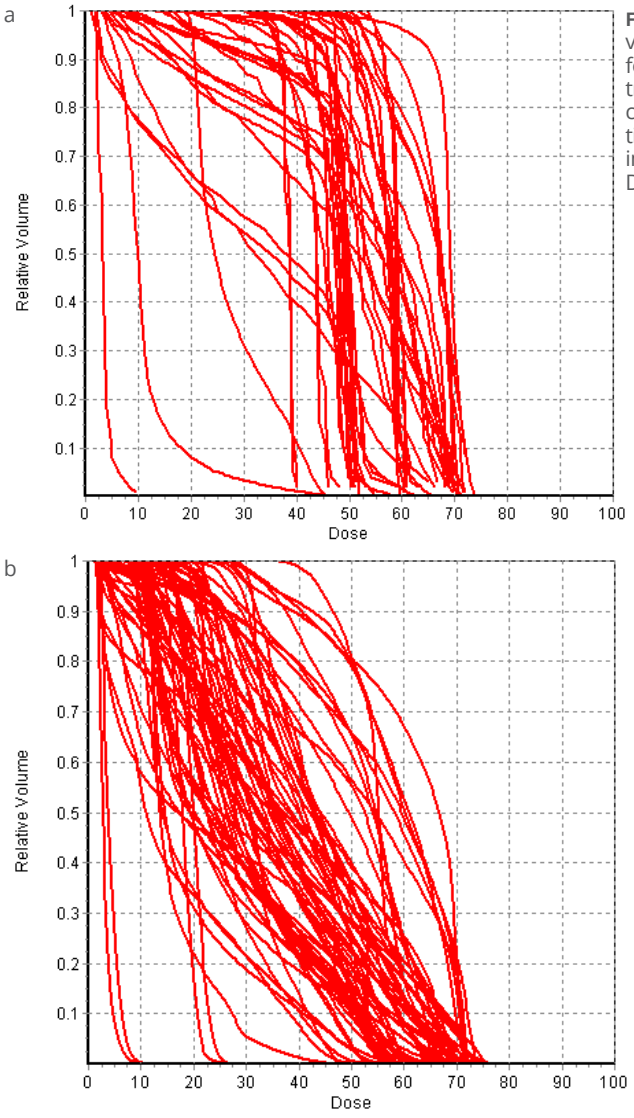

Figure 3. Cumulative dose-volume histograms (DVHs) for parotid glands in patients treated for oropharyngeal cancer with either conventional radiotherapy (a) or intensity-modulated RT (b). Dose in Gy.

intact. Radiation damage to the extracellular environment (excretory ducts, blood vessels, nerves) could be important in late parotid gland functional loss due to secondary effects [16,27]. For rat parotid gland, a clear region-dependent radiosensitivity was found related to the presence of these supplying structures in the radiation field. It was suggested that the mean dose concept has limited use in the prediction

of late effects [16]. The extrapolation of these results to humans, however, is difficult already on the basis of different anatomical relationships. In this study, the mean dose described similar late effects (at one year) independent of treatment technique and related dose distributions.

Extending this conclusion, the use of different radiation techniques provides no explanation for the differences in tolerance dose at one year after RT previously published for the parotid gland [6,7]. We found no evidence of a threshold dose in IMRT- or CRT-treated patients at any timepoint. Discrepancies between the dose-response curve initially published by Eisbruch *et al.* and the curve published by our group may stem from differences in the underlying data. The number of patients in the critical mean dose range of 30-40 Gy was limited in the study by Eisbruch *et al*; possibly influencing the threshold shape of the resulting dose-response curve. High intra-individual variation in parotid flow measurements (up to 45%) can also explain some variation in the resulting curve [7,28,29].

In a recent update of the data presented by Eisbruch *et al*; sophisticated statistical modelling is used to describe dose-response relationships. Li *et al.* [8] use the complete range of salivary flow measurements in their analysis and do not dichotomize the data on the basis of a complication definition. Rather than an absolute threshold, a steep decline was found in post-RT saliva production in the mean dose range of 25-35 Gy. Based on their model, a mean dose of 38 Gy (equal to our TD_{50} for IMRT-treated patients at one year post-RT) would lead to a stimulated flow ratio of 7% one month post-RT and 35% of baseline at *two* years post-RT. Still, there are relative few data in the 30-40 Gy mean dose range. A clear comparison between the updated report by Li *et al.* and the results presented here can, however, not be made. We previously found the Lyman model an acceptable fit for the parotid flow data in the first 108 conventionally treated patients, also described in this study [7]. The number of predicted complications was in agreement with the observed number of complications at all timepoints post-RT.

In NTCP-modelling, defining a threshold value for parotid flow measurements can influence the shape of the NTCP curve [16]. We have recently investigated several cut-off points for the best definition

for objective parotid gland toxicity. For stimulated parotid flow measurements using Lashley cups and for scintigraphy data, reduction of parotid salivary flow to <25% of the pre-radiotherapy output (EORTC/RTOG grade 4 xerostomia) was found an optimal threshold [13]. For that reason, this complication definition was also used in this study.

Several dosimetric factors were not taken into account in this analysis. With CRT the treatment fields were mainly designed using radiographs, in contrast to CT-guided target definition in IMRT. The resulting difference in targeted volumes may have had an additional influence on dose-distributions in the parotid gland. Second, conventional fractionation was used as well as integrated boost techniques and hyperfractionated RT. These altered fractionation schedules and the resulting variation in biologically equivalent dose may affect dose-response relationships in the parotid gland [14,30]. The α/β ratio for early and late effects may also be different, leading to different sensitivity to fraction size [14]. Excluding the patients that received hyperfractionated RT, along with those that received chemotherapy or brachytherapy, did not change our final conclusions.

Finally, the volume-dependency parameter n of the Lyman equation was fixed at 1. The results from this study indicate that for early effects, a value of parameter n other than 1 is needed. Previously high values for n (equal to or higher than 1) have been described, assuming a large volume effect related to the parallel architecture of the parotid glands [6,7,14]. The mean dose (n = 1), in contrast to certain partial volume thresholds, was found to be a significant independent factor in predicting post-RT parotid gland function. Mean dose and partial volumes were, however, highly correlated [6]. In a detailed study by Blanco *et al.* [5] the one-parameter mean dose-exponential model also provided a good description of stimulated saliva flow rates at 6 and 12 months post-RT. This was regardless of treatment delivery by IMRT or 3D-conformal RT, determined using logistic regression modelling. The latter group in that study was probably too small (14 patients) to detect a possible difference between treatment modalities. This is the first clinical study to demonstrate the limitations of the mean parotid gland dose in describing parotid gland function early (up to 6 months) post-RT.

In conclusion, one year after radiotherapy, mean dose based NTCP-curves for CRT and IMRT are comparable with a TD_{50} of 40 and 38 Gy, respectively. Early after radiotherapy (up to 6 months) mean dose based (Lyman) models failed to fully describe the effects of radiotherapy on the parotid glands.

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3 Parotid gland function after radiotherapy: the combined Michigan and Utrecht experience

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Abstract

Purpose: To analyze the combined and updated results from the University of Michigan and University Medical Center Utrecht on normal tissue complication probability (NTCP) of the parotid gland at 1 year after radiotherapy (RT) for head and neck head-and-neck cancer (HNC).

Patients and methods: 222 prospectively analyzed patients with various HNC were treated with conventional and intensity-modulated RT. Stimulated individual parotid gland flow rates were measured before RT and 1 year after RT using Lashley cups at both centers. A flow ratio <25% of pre-treatment was defined as a complication. The data were fitted to the Lyman-Kutcher-Burman (LKB) model.

Results: A total of 384 parotid glands (Michigan: 157; Utrecht: 227 glands) was available for analysis at 1 year after RT. Combined NTCP analysis based on mean dose resulted in TD_{50} (uniform dose leading to 50% complication probability) of 39.9 Gy and m (steepness of the curve) of 0.40. The resulting NTCP curve had good qualitative agreement with the combined clinical data. Mean doses 25-30 Gy were associated with 17-26% NTCP.

Conclusions: A definite NTCP curve for parotid gland function at 1 year after RT is presented based on mean dose. No threshold dose was observed and the TD_{50} was equal to 40 Gy.

Introduction

Radiotherapy (RT) for head-and-neck cancer (HNC) generally results in a high radiation dose to the major salivary glands. Reduced salivary flow leads to xerostomia and this is a major cause of decreased quality of life in HNC survivors [1]. The relationship between radiation dose, irradiated volume and the resulting salivary function after RT has been extensively studied for the parotid salivary glands.

In these glands, a strong correlation exists between the mean dose to the gland and residual post-RT function [2]. The University of Michigan and the University Medical Center Utrecht have published normal tissue complication probability (NTCP) curves that were based on a large cohort of patients [3, 4]. Both have used objective parotid salivary flow measurements (using Lashley cups) as a function of the mean dose to study NTCP-parameters. However, for parotid gland function one year after RT, different NTCP-parameters were obtained. Eisbruch *et al.* described steep dose-response relationships in a population of 88 HNC patients treated with an intensity-modulated radiotherapy (IMRT) technique [3]. The TD_{50} (the dose which results in 50% complication probability for whole parotid gland irradiated uniformly) at one year was determined at 28 Gy. Roesink *et al.* found no threshold dose in a study of 108 HNC patients treated with conventional radiotherapy (CRT) using mostly opposed lateral photon beams [4]. The TD_{50} at one year in that study was equal to 39 Gy. These differences could have been caused by the use of different RT techniques. Recently however, it was shown that NTCP-parameters for CRT and IMRT one year post-RT are comparable: TD_{50} being equal to 40 and 38 Gy, respectively [5].

The aim of this study was to analyze the combined, updated results from both institutions in order to arrive at a definitive NTCP curve for parotid gland function one year after RT and to guide clinical decision making.

Patients and methods

Patients and radiotherapy

At the University of Michigan, 92 HNC patients treated with primary or postoperative RT between 1994 and 2005 were prospectively evaluated. The parotid gland data (one year post-RT) of the first 54 patients were published previously [3]. The remaining 38 patients were also described earlier [6], however not with respect to parotid gland function post-RT. Patients were treated using forward-planned, inverse-planned and beamlet-IMRT according to previously detailed methods [3,6,7]. The prescribed dose to the gross tumor volume or dissection site was 60-75 Gy in 1.8-2.0 Gy fractions (5 days per week).

In Utrecht, a total of 130 HNC patients were prospectively analyzed between 1996 and 2007. These patients' parotid gland function data (up to one year after RT) were published recently [5]. CRT (using opposing lateral beams) was used as well as inverse-planned IMRT for the primary or postoperative treatment of various HNC. Details on treatment planning and target delineation have been published previously [4,8]. The prescribed dose to the gross tumor volume or postoperative tumor bed was 50-70 Gy in 2 Gy fractions using CRT and 69-70 Gy in 2.0-2.3 Gy fractions with IMRT (5 days per week).

For each patient, contrast-enhanced computer-tomography (CT) imaging of the HN region was performed. The left and right parotid glands were delineated on the axial CT slices. Three-dimensional dose distributions in the complete volume of the parotid glands were calculated and converted into dose-volume histograms (DVHs). Each gland was thus analyzed separately.

These prospective studies were respectively approved by the Institutional Review Board of the University of Michigan and the Medical Ethical Committee of the University Medical Center Utrecht. Informed consent was obtained from each patient.

Parotid flow measurements

Techniques that were used in Michigan and Utrecht for parotid saliva measurements have been described previously [3,4]. The stimulated parotid saliva was collected by Lashley cups after applying citric

acid solution (2-5%) on the mobile part of the tongue. Patients were instructed not to eat or drink 60-90 minutes before saliva collection. To avoid the influence of diurnal variation in salivary flow, consecutive measurements were scheduled at the same daytime in each patient. Salivary flow rates were measured before treatment and at one year after RT. The flow rate at one year was converted into the percentage of the baseline flow rate. A complication was defined for each individual gland as a stimulated parotid flow ratio <25% of the pre-treatment flow rate, or grade 4 xerostomia according to the Radiation Therapy Oncology Group/ European Organization for Research and Treatment of Cancer (RTOG/ EORTC) Late Effects Consensus Conference [9].

Normal tissue complication probability model

The parotid flow data were fitted to the NTCP-model proposed by Lyman, Kutcher and Burman (LKB-model) [10,11]. This model is assumed to quantitatively establish the effects of both radiation dose and irradiated volume on the probability of radiation-induced changes in parotid gland function. Three parameters are present in the sigmoid dose-response relationship described in the LKB-model. TD_{50} is the dose at which a 50% complication probability is seen after uniform parotid gland irradiation and parameter *m* describes the slope of the NTCP-curve. Parameter *n* accounts for the volume effect of an organ and depends on the tissue organization [10]. If *n* is high (close to or higher than 1) partial sparing of the organ reduces complication probability. This is referred to as a parallel organization of the organ, such as in liver and lungs. The mean dose influences complication probability in this situation. If *n* approaches zero, the maximum dose influences complication probability. This serial architecture is thought to be present in spinal cord and esophagus, for example.

NTCP-curves that were published previously have used the mean dose ($n = 1$) as descriptive dose parameter [3-5]. In the present combined analysis, we also fixed *n* at 1 and thereby described parotid gland function one year after RT as a function of the mean dose (mean dose model). In addition, we fitted the combined data to the LKB-model with *n* unrestricted in order to investigate if a *n* value other than 1 described the data better (full LKB-model).

Statistical analysis

Patient characteristics were analyzed using descriptive statistics (mean, ranges or proportions; where appropriate). To investigate whether both institutes differed with respect to the NTCP endpoint one year after RT, the relative risk (RR) and accompanying 95% confidence interval (CI) of parotid gland complication probability was calculated for Utrecht versus Michigan. A modified Poisson regression model was used to adjust this relative risk for the mean parotid gland dose [5, 12]. The NTCP-parameters (TD_{50}, n and m) were determined by a maximum likelihood estimation method described previously [4,13]. Before combining the data, separate analyses were performed for the Utrecht and Michigan cohort. To compare the fits of the mean dose model (n = 1) and the full LKB-model (n unrestricted) we computed the goodness-of-fit (GOF) using the deviance (D). This parameter is defined as minus twice the difference between the log likelihood of the actual fitted model and the log likelihood of the experimental data [14].

All analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA) except for the modified Poisson regression analysis that was performed using the PROC GENMOD procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The two-tailed significance level was set at 0.05. The NTCP-modelling was performed using software developed at the Department of Radiation Oncology at the University Medical Center in Groningen, The Netherlands [13].

Results

Patient, tumor and treatment characteristics of the patients are outlined in *Table 1*. In total, 384 parotid glands (Michigan: 157; Utrecht: 227) from 222 patients were available for analysis one year after RT. The patient population in both Michigan and Utrecht included heterogeneous tumor sites in order to represent the full range of the mean dose in the parotid gland, which is optimal for NTCP-modelling (*Figure 1*).

Table 1 Patient and tumor characteristics, n (%).

	Michigan *(n = 92)*	**Utrecht** *(n = 130)*
Gender		
Male	65 (71)	92 (71)
Female	27 (29)	38 (29)
Age		
Median	54	58
Range	20-82	24-99
Tumor site		
Larynx	4 (4)	47 (36)
Hypopharynx	4 (4)	2 (2)
Oropharynx	53 (58)	41 (31)
Nasopharynx	4 (4)	12 (9)
Oral cavity	8 (9)	11 (8)
Nasal cavity		6 (5)
Salivary glands	9 (10)	1 (1)
Unknown primary	4 (4)	1 (1)
Other *	6 (7)	9 (7)
Stage (AJCC)		
I	2 (2)	12 (9)
II	12 (13)	35 (27)
III	26 (29)	30 (23)
IV	48 (52)	36 (28)
Recurrent/ unknown	4 (4)	17 (13)
Radiotherapy		
Definitive	49 (53)	85 (65)
Postoperative	43 (47)	45 (35)

Abbreviation: AJCC = American Joint Committee on Cancer Staging Manual (6th edition, 2002).
* Other: (Michigan) skin: 4 patients and maxillary sinuses: 2 patients; (Utrecht) Hodgkin/ non-Hodgkin lymphoma: 4 patients, skin/ lip: 3 patients, orbita: 1 patient and upper trachea: 1 patient.

The Michigan and Utrecht cohorts did not differ with respect to parotid gland complication probability one year after RT, corrected for mean dose (*Table 2*). Comparable NTCP-parameters for both cohorts with overlapping confidence intervals were observed (*Table 3*). Consequently, the data could be combined to arrive at a single set of NTCP-parameters.

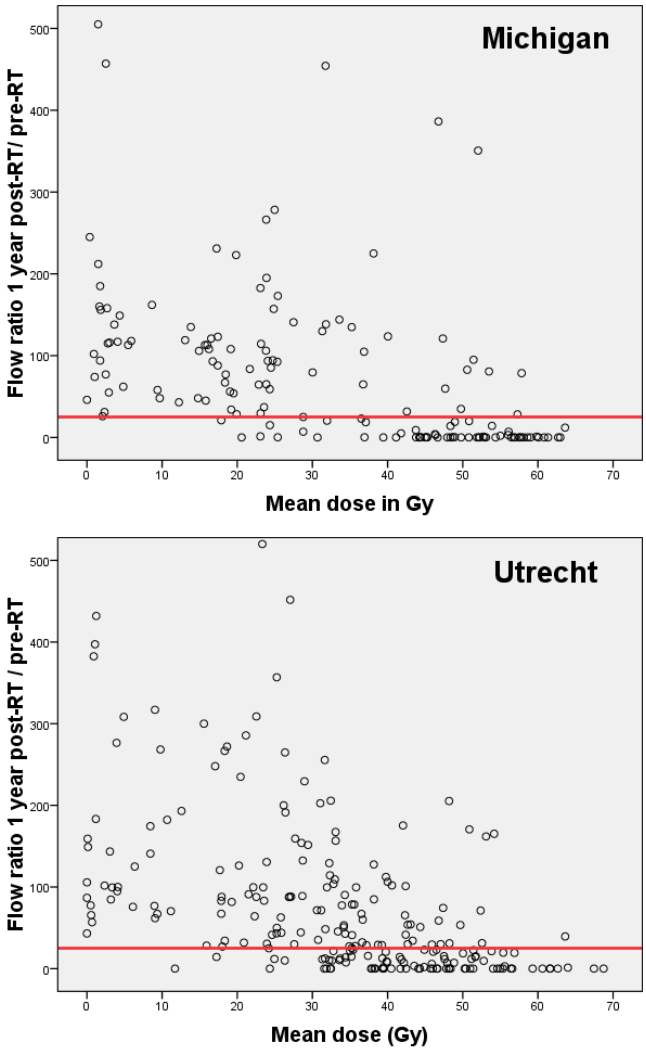

Figure 1 Parotid flow ratio at one year post-RT as a function of the mean parotid gland dose for Michigan (157 glands) and Utrecht (227 glands). The horizontal line indicates the complication threshold according to RTOG/ EORTC grade 4 xerostomia (flow ratio <25% of pre-treatment).

The combined analysis according to the mean dose model (n = 1) resulted in TD_{50} = 39.9 Gy and m = 0.40. Fitting of the full LKB-model (n unrestricted) yielded similar results. Volume dependency parameter n equaled 1.13 in the optimal LKB-model fit. This indicates a parallel organization of functional subunits in the parotid gland. There was hardly any difference in goodness-of-fit between both models

(expressed as the deviance (*D*), *Table 4*). At one year post-RT, the mean dose described the probability of flow complications very satisfactory. We chose to describe the NTCP by the mean dose model over the full LKB-model because only two parameters had to be fitted (TD_{50} and *m*) with comparable goodness-of-fit. Furthermore, it is easy to use in treatment planning. Very similar NTCP-curves were observed for the Michigan and Utrecht cohorts separately, confirming the data can be combined (*Figure 2*). The combined NTCP-curve based on mean dose (*Figure 3*) had good qualitative agreement with the clinical data.

Table 2 Poisson regression analysis for the risk of parotid flow complications one year after RT for Michigan versus Utrecht, corrected for the mean parotid gland dose.

One year post-RT	Crude RR (95% CI)	Adjusted RR (95% CI) *	*p*-value †
Michigan vs Utrecht	1.01 (0.79-1.3)	0.96 (0.78-1.18)	0.68
Mean dose (Gy)		1.05 (1.04-1.06)	<0.0001

Abbreviations: 95% CI = 95% confidence interval; RR = relative risk.
* Adjusted for mean parotid gland dose (in Gy) on the endpoint parotid flow to <25% of pre-treatment.
† *p*-value for the adjusted RR.

Table 3 Parameters TD_{50} and *m* (95% CI) in terms of mean parotid gland dose (*n* = 1) for flow data one year post-RT.

	Michigan	Utrecht
TD_{50} (Gy)	40.5 (36.8-44.1)	39.7 (37-43.3)
m	0.36 (0.28-0.44)	0.44 (0.35-0.54)

Abbreviations: CI = confidence interval; TD_{50} = the uniform dose to the whole organ resulting in 50% complication probability; *m* = slope of the NTCP curve.

Table 4 Combined analysis: parameters TD_{50}, *m* and *n* (95% CI) in terms of mean dose (*n* = 1) and with *n* unrestricted for flow data one year post-RT. Goodness-of-fit is expressed as the deviance (*D*).

	Mean dose (*n* = 1)	Full LKB (*n* unrestricted)
TD_{50} (Gy)	39.9 (37.3-42.8)	39.4 (33.8-41.8)
m	0.40 (0.34-0.51)	0.42 (0.36-0.58)
n	1	1.13 (0.75-14.3)
D	339.2	340.6

Abbreviations: CI = confidence interval; TD_{50} = the uniform dose to the whole organ resulting in 50% complication probability; *m* = slope of the complication probability curve; *n* = volume dependency parameter.

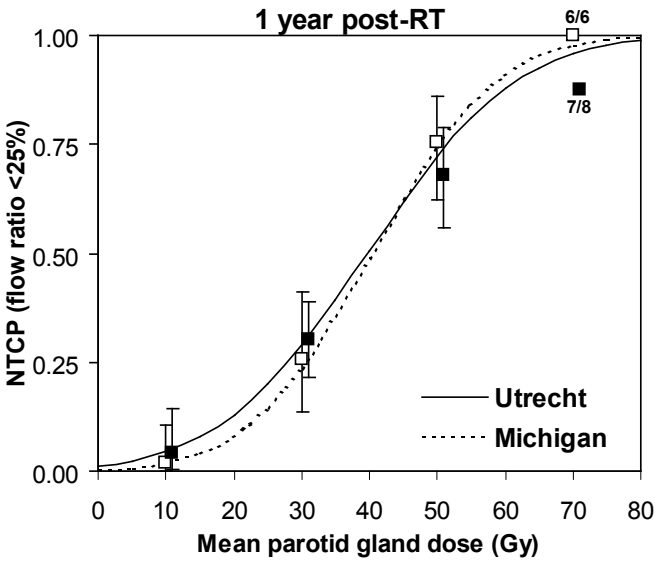

Figure 2 Normal tissue complication probability (NTCP) curves as a function of the mean parotid gland dose for Michigan (dashed line) and Utrecht (solid line). Clinical NTCP values (using mean dose bins of 20 Gy) are shown for Michigan (open squares) and Utrecht (black squares) including 95% CI.

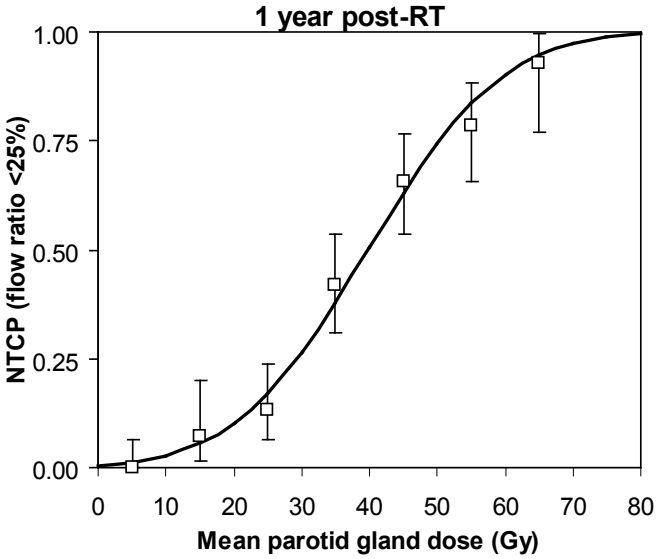

Figure 3 Combined Michigan and Utrecht normal tissue complication probability (NTCP) curve as a function of the mean parotid gland dose. Clinical NTCP values (using mean dose bins of 10 Gy) are shown including 95% CI.

Discussion

This study represents the largest series in literature of selective parotid gland function measurements one year after radiotherapy for HNC. Based on this analysis, a definite NTCP curve for parotid gland function (one year after RT) is presented for use in clinical practice. No threshold dose was observed. At a mean dose of 39.9 Gy, there is a 50% probability of parotid gland flow reduction to <25% of the pre-radiotherapy flow rate. The resulting NTCP-parameterization of the combined clinical data is consistent with the presumed parallel organization in the parotid gland. Also, it shows the strong predictive ability of the mean dose on the probability of parotid flow reduction to <25% at one year.

This report represents an update of the data presented by Eisbruch *et al.* and by Roesink *et al.* [3,4]. Additional patients were included in salivary gland studies at both departments and more advanced RT-techniques such as inverse-planned IMRT were used [5,7]. The results therefore represent a heterogeneous HNC patient population (n = 222) treated with both conventional 3D and IMRT-techniques. Differences with previously published results probably stem from inhomogeneity in the underlying data, especially in the critical dose range (30-40 Gy). The initial Michigan dataset [3] had little data (n = 3) in the 30-40 Gy mean dose range (near the TD_{50}) at one year. This probably influenced the Lyman model fit and the steep shape of the resulting NTCP-curve. The current updated Michigan dataset (*Figure 1*) however, contains more data in the critical mean dose range and the individual NTCP-curves from both institutions are very similar (*Figure 2*). Mean parotid gland doses of 25-30 Gy now correspond to 17-26% complication probability one year post-RT. Taken altogether, a large cohort of patients and measurements are required to reliably describe the dose-response curve in the parotid gland and in any other organ, for that matter [17]. Combining multi-institutional experience is one way to achieve this.

There have been several publications on dose-response modelling in the parotid gland using whole salivary flow and salivary gland scintigraphy in stead of selective parotid flow measurements [18-21]. These studies

have often used different endpoints which makes it difficult to compare them. Besides the fact that most data originates from relatively small patient cohorts, there are some drawbacks to the techniques mentioned. With whole mouth saliva, individual parotid flow cannot be measured and uncertainty is introduced as the contributions from the submandibular and minor salivary glands are ignored. Scintigraphy is a good indicator of parotid gland function and can detect the gland's ability to collect and excrete saliva to small amounts. It is expensive however, more invasive and requires hospital equipment. Moreover, in a comparison to determine the best measure for parotid gland function, we found that stimulated flow measurements one year after RT using Lashley cups (complication defined as flow <25% of the pre-RT output) correlated better with mean parotid gland dose than did scintigraphy [22].

In conclusion, when aiming at preservation of parotid gland function after RT for HNC, this study shows a gradual increase in NTCP with increasing mean dose. In fact, a treatment planning constraint of 25-30 Gy corresponds to 17-26% complication probability at one year. At 40 Gy mean dose, there is a 50% probability of parotid gland flow reduction to <25% of the pre-RT flow (*Figure 3*). By combining multi-institutional experience we obtained a large patient cohort which helped to construct a reliable NTCP-curve for use in RT practice.

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4 Xerostomia: a day and night difference

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Abstract

Purpose: To compare patient-reported xerostomia during daytime and during nighttime with objectively measured parotid and submandibular gland function in a cohort of head-and-neck cancer (HNC) patients treated with RT.

Patients and methods: A cohort of 138 HNC patients underwent objective measurements of parotid (PF) and submandibular (SMF) gland function and completed a xerostomia questionnaire (XQ) before RT, at 6 weeks, 6 months and one year after RT. No attempt was made to spare the submandibular gland(s). The XQ contained specific questions concerning the sensation of dry mouth during day- (XD) and nighttime (XN), scored on a 5-point Likert scale. Patients with no or mild (grade 1-3) xerostomia and patients with more severe (grade 4-5) complaints were grouped together.

Results: Before RT, no association existed between dry mouth complaints and PF or SMF. At 6 weeks, 6 months and one year after RT; 37%, 51% and 36% had grade 4-5 XD and 65%, 64% and 56% had grade 4-5 XN, respectively. Patients with grade 4-5 XD and XN had significant worse SMF at all timepoints after RT compared with patients with grade 1-3 XD and XN, while PF was significantly worse only at 6 weeks after RT. In multivariate analyses, SMF was consistently the most important factor related to XN after treatment. PF significantly influenced XD up to one year after RT.

Conclusions: Differentiating between complaints during day- and nighttime in xerostomia research is necessary. Dry mouth at night is a frequent problem after (parotid-sparing) RT for HNC and is explained by submandibular gland dysfunction. Sparing of the contralateral submandibular gland, in addition to parotid gland sparing, may result in improved patient-reported xerostomia.

Introduction

Radiotherapy (RT) for head and neck cancer (HNC) generally results in high radiation doses to the major salivary glands. The resulting decrease in salivary flow leads to xerostomia and this has a major impact on quality of life in HNC survivors [1,2]. Sparing of the parotid glands using intensity-modulated radiotherapy (IMRT) is feasible and significantly improves parotid gland flow over time in patients treated for HNC [3]. The impact on patient-reported xerostomia remains unclear however. The recent PARSPORT trial showed a significant decrease in patient-reported xerostomia following parotid gland sparing [4]. However, Kam *et al*. did not show a synchronous improvement in patient-reported xerostomia in a cohort of patients with nasopharyngeal carcinoma randomized to IMRT or conventional RT [5].

The submandibular glands are responsible for most saliva production (60-65%) in the non-stimulated state [6]. During sleep, salivary flow rate is low and originates mainly from the submandibular glands [7,8]. Although HNC patients with xerostomia frequently complain of dry mouth at night, there have been no reports in the literature showing a correlation with submandibular gland function. In contrast to the serous secretion from the parotid glands, the submandibular glands produce a mixed serous and mucous saliva. The mucins herein function as mucosal lubricants that bind water and help to keep the mucosal surfaces in the oral cavity in a hydrated state [9]. Through this mechanism, salivary mucins could have a significant impact on the patient's subjective sensation of moisture. It might also explain the discrepancy between preserved parotid flow and the relative lack of improvement in patient-reported xerostomia.

The aim of this study was to compare patient-reported xerostomia throughout day and night with objective, selectively measured parotid and submandibular gland function in a cohort of HNC patients treated with RT. Because of the mentioned physiologic and diurnal variations in quantity and quality of parotid and submandibular saliva, the function of these major salivary glands could have a different impact on xerostomia.

Patients and methods

Patients and radiotherapy

All data were gathered prospectively. In total, 138 patients with HNC were consecutively included in salivary function studies at our department. Conventional RT (CRT) was applied in 46 patients with mainly laryngeal and oropharyngeal cancer that participated in a double-blind, placebo-controlled, randomized clinical trial investigating the effect of pilocarpine on radiation-induced xerostomia [10]. Only the patients that received placebo were included in this analysis. Details on RT treatment planning have been reported previously [3,11]. The prescribed dose to the gross tumor volume (GTV) or postoperative tumor bed was 50-70 Gy in 2 Gy fractions, using mainly opposing lateral photon beams.

After the introduction of IMRT at our department, another 92 patients were included in prospective studies on parotid gland sparing RT. Details on treatment planning and delineation have been published elsewhere [11,12]. Depending on the concomitant use of chemotherapy, 69 Gy in 30 fractions (simultaneous boost) or 70 Gy in 35 fractions (sequential boost; with chemotherapy) was prescribed to the GTV. Along with the target volumes, the organs at risk (OAR) including the parotid glands were delineated on the planning CT scan. Inverse planned, step-and-shoot IMRT was applied with the intention to spare *both* parotid glands. No attempt was made to spare the (contralateral) submandibular gland(s). All studies described were approved by the Medical Ethics Committee of the University Medical Center Utrecht. Informed consent was obtained from each patient.

Parotid and submandibular flow measurements

Techniques that were used for parotid saliva measurements have been described previously [3,11]. Stimulated salivary flow rates were mea-sured before treatment, 6 weeks, 6 months and at one year after RT. Patients were instructed not to eat or drink 60 minutes before saliva collection. Stimulated parotid saliva was collected for 10 minutes using Lashley cups after applying citric acid solution (5%) on the mobile part of the tongue every 60 seconds. For the purpose of this study,

saliva from the left and right parotid gland was added together at each timepoint (parotid gland flow; PF).
At the same time, saliva near Wharton's duct orifices was collected by gentle suction with a micropipette. It represents predominantly salivary flow from the submandibular glands (SMF) but also varying amounts from the sublingual glands. The collected samples were weighted and converted to ml/ min assuming the specific gravity of saliva to be 1.0 g/ml. To avoid the influence of diurnal variation in salivary flow, consecutive measurements were scheduled as much as possible at the same daytime for each patient.

Assessment of patient-reported xerostomia

At the same timepoints at which saliva was collected, all patients were asked to complete a xerostomia questionnaire (XQ). The XQ contains 12 questions and was developed at the department of Oral and Maxillofacial Surgery of the University Medical Center Groningen to evaluate the use of saliva substitutes in patients with xerostomia [13,14]. It was also used in a double-blind, randomized clinical trial investigating the effect of pilocarpine on radiation-induced xerostomia [10]. The XQ contains questions related to xerostomia (dry mouth during day- and nighttime, eating, speaking, swallowing, sleeping) and is scored on a 5-point Likert scale. A score of '1' means no complaints, while a score of '5' implies complaints are always present.
For the purpose of this study, we were interested only in the two questions addressing the sensation of dry mouth during day- ('Do you have a dry mouth during the day') and nighttime ('Do you have a dry mouth at night'). In the current analysis, we dichotomized xerostomia into 'severe' (grade 4-5) or 'none-to-mild' (grade 1-3). This was done for the symptom score during daytime (XD) and at night (XN). To explore the data, patients were grouped together according to the pattern of complaints at day- and nighttime. Group A consisted of patients with no or mild complaints (grade 1-3) during day- and nighttime. Patients in group B had no or mild complaints during the day (grade 1-3) but had severe complaints at night (grade 4-5). Group C-patients had severe complaints (grade 4-5) during daytime and at night. The combination

of severe complaints during daytime (grade 4-5 XD) and no or mild complaints at night (grade 1-3 XN) occurred rarely (n = 3).

Statistical analysis

Baseline patient characteristics were reported using descriptive statistics (mean, median, ranges or proportions; where appropriate). Differences in parotid and submandibular gland flow according to the pattern of complaints (for *Figure 2*) were analyzed using the Mann-Whitney *U* test. Correlations were calculated using Pearson's correlation coefficient (*r*).

Patients that were lost to follow-up, missed their appointment or had recurrent illness were blinded in the analysis.

To investigate the (independent) association between PF/ SMF and xerostomia at day or night at three timepoints after RT (6 weeks, 6 months and one year), we performed multivariate logistic regression analyses. The outcome in these analyses was the presence or absence of severe xerostomia. Two different models were constructed. The first model was adjusted for age, sex and for the potential confounding effect of surgery (including neck dissection), chemotherapy and the baseline parotid or submandibular flow (ml/ min). In the second model, model 1 was mutually adjusted for SMF or PF depending on the parameter of interest. This second and final model was constructed, since we were specifically interested in the independent influence of parotid and submandibular saliva on xerostomia complaints during day- or nighttime. The odds ratio's (OR's) and corresponding 95% confidence intervals (95% CIs) were calculated. All analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). A p value of <0.05 was considered statistically significant.

Results

The study population (n = 138) included mainly patients who received primary radiotherapy for their HNC. The oropharynx was the predominant tumor site (*Table 1*). In 25 patients (18%), an ipsilateral neck dissection was performed including the ipsilateral submandibular gland. Before RT, the median PF was 0.21 ml/ min (interquartile range

0.10-0.41) and the median SMF was 0.35 ml/ min (interquartile range 0.17-0.58). This corresponds to 38% versus 62% of the whole saliva output, respectively (p = 0.0001 using the Wilcoxon signed-ranks test). The mean dose to the PGs was much lower compared with the SMGs (36.5 versus 59.8 Gy, respectively). Significant correlations existed

Table 1 Patient and tumor characteristics, n (%).

Gender	
Male	89 (65)
Female	49 (35)
Age (median; range)	59 (35-88)
Tumor site	
Larynx	25 (18)
Hypopharynx	6 (4)
Oropharynx	83 (60)
Nasopharynx	17 (13)
Oral cavity	6 (4)
Unknown primary	1 (1)
T stage	
T1	28 (21)
T2	61 (44)
T3	25 (18)
T4	21 (15)
Tx	3 (2)
N stage	
N0	60 (44)
N1	24 (17)
N2a-b	38 (28)
N2c	13 (9)
N2 nasopharynx	3 (2)
RT modality	
IMRT	92 (67)
CRT	46 (33)
Mean dose (Gy; range)	
PG	36.5 (3.1-68.7)
SMG	59.8 (17.5-72.5)
Surgery	
Tumor ± iND	17 (12)
iND only	12 (9)
None	109 (79)
Chemotherapy	19 (14)

Abbreviations: IMRT = intensity modulated radiotherapy; CRT = conventional RT; Gy = dose in Gray; PG = parotid gland; SMG = submandibular gland; iND = ipsilateral neck dissection.

between glandular mean dose and function: mean PG dose and PF were correlated at 6 weeks, 6 months and 1 year after RT (Pearson *r* = -0.41, -0.37 and -0.38; respectively) while mean dose to the contralateral SMG showed the strongest correlations with SMF at 6 months and 1 year after RT (Pearson *r* = -0.03, -0.33 and -0.52; respectively).

At 6 weeks after RT, a total of 117 patients were available for analysis, at 6 months 94 patients and at one year 82 patients. Pre-RT, grade 4-5 complaints of xerostomia were reported by 5% and 20% of the patients, at day- and nighttime respectively. At 6 weeks, 6 months and one year after RT; 37%, 51% and 36% had grade 4-5 XD and 65%, 64% and 56% grade 4-5 XN, respectively (*Figure 1*). The overall percentage of patients with severe complaints (XQ grade 4-5) at daytime (XD) and/ or at night (XN) is virtually unchanged at one year after RT (65%). The high incidence of severe dry mouth at night (grade 4-5 XN) also affected sleeping in patients. Patients with severe dry mouth at night 12 months after RT reported significant more sleeping problems compared with patients without severe dry mouth at night, with 19% versus 2% reporting sleeping disturbance grade 4-5 as measured with the XQ respectively (p = 0.021 using t-test).

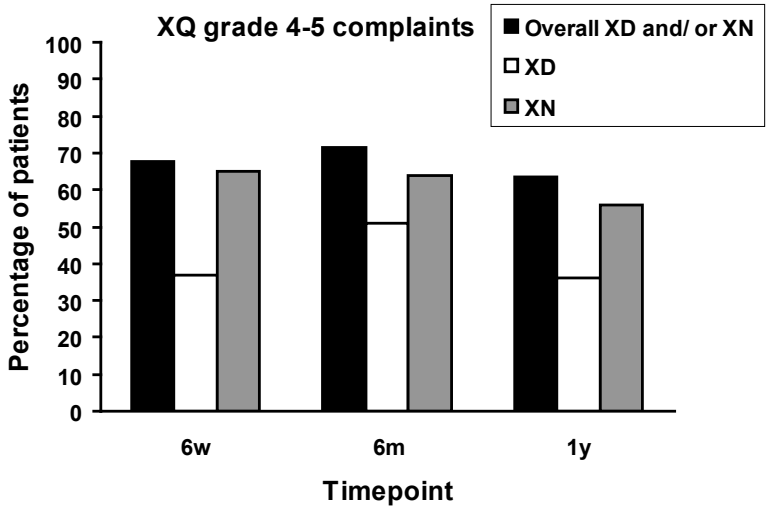

Figure 1. Severe patient-reported xerostomia: the percentage of patients with severe complaints of dry mouth (XQ grade 4-5) during daytime (XD) and/ or at night (XN) at different timepoints after RT.
Abbreviations: 6w = 6 weeks after RT; 6m = 6 months after RT; 1y = 1 year after RT.

Using the XQ, three main patient groups were distinguished, according to their pattern of complaints (*Figure 2*). The group with grade 4-5 XD and grade 1-3 XN (n = 3) was omitted in the figure. After RT, PF improved continually over time, irrespective of the pattern of complaints.

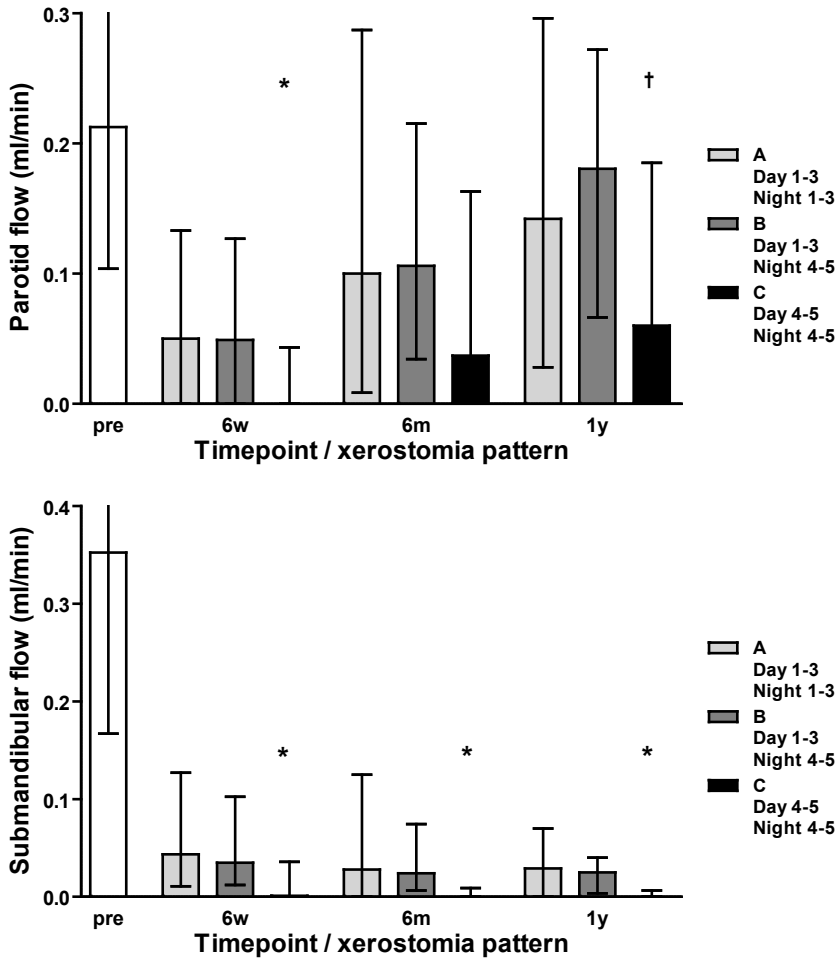

Figure 2. Grouped analysis of parotid and submandibular gland flow (median; interquartile range) in patients according to their pattern of complaints at 6 weeks, 6 months and one year after RT.
***** p <0.05 for the difference between group C and group A and group C and B (Mann-Whitney *U* test).
† p <0.05 for the difference between group C and group B (Mann-Whitney *U* test).
Abbreviations: pre = before RT; 6w = 6 weeks after RT; 6m = 6 months after RT; 1y = one year after RT; day 1-3 = grade 1-3 complaints at daytime; day 4-5 = grade 4-5 complaints at daytime; night 1-3 = grade 1-3 complaints at night; night 4-5: grade 4-5 complaints at night.

SMF on the other hand, decreased after RT in all groups with a tendency to stabilize at one year. The mean SMF was significantly lower in group C compared with groups A and B, at all timepoints after RT. The mean PF only differed significantly at 6 weeks in group C compared with groups A and B. Groups A and B did not differ significantly from each other with respect to the mean PF and mean SMF at any timepoint after RT.

Table 2 shows the results of the multivariate logistic regression analyses. Before RT, no association existed between dry mouth complaints and PG or SMG. At 6 weeks after RT, XD was significantly related to PG and SMG (model 1). After adjustment for PG, the association of SMG with xerostomia weakened and lost significance. SMG appeared the most important determinant of XN although borderline significant (OR = 0.62; $p = 0.08$). At 6 months, for XD, both PG and SMG were significantly associated with xerostomia complaints (model 1). SMG appeared as the most important determinant in model 2 (OR = 0.03 with 95% CI 0.0-0.36). For XN at 6 months, SMG was the strongest explanatory variable (OR = 0.34 with 95% CI 0.13-0.89). Twelve months after RT, only PG was significantly associated with XD (OR = 0.66 with 95% CI 0.44-0.97). In contrast, XN at one year was only associated with SMG (OR = 0.45 with 95% CI 0.21-0.98). For PG, odds ratio's were close to one.

Whole saliva (PF + SMF) showed a very strong correlation with parotid gland function after RT (Pearson $r > 0.8$ at all timepoints) and was therefore not included in the analyses. Entering whole saliva into the multivariate analyses instead of PF and SMF alone did not yield a stronger determinant for XD or XN (data not shown).

Table 2 Multivariate logistic regression analyses of factors related to the sensation of dry mouth during day- and nighttime (grade 1-3 vs 4-5) at 6 weeks, 6 months and one year after RT (OR ± 95% CI).

Factor	MVA		
	Model 1*	Model 2†	*p*-value§
Pre-RT			
XD			
PF	0.73 (0.43-1.24)	0.72 (0.41-1.25)	ns
SMF	1.01 (0.81-1.26)	1.05 (0.84-1.30)	ns
XN			
PF	0.91 (0.76-1.10)	0.93 (0.77-1.12)	ns
SMF	0.97 (0.86-1.10)	0.98 (0.86-1.11)	ns
6 weeks			
XD			
PF	0.41 (0.22-0.77)	0.38 (0.17-0.83)	0.016
SMF	0.37 (0.16-0.83)	0.55 (0.25-1.24)	ns
XN			
PF	0.95 (0.65-1.39)	1.05 (0.69-1.59)	ns
SMF	0.61 (0.36-1.02)	0.62 (0.36-1.06)	0.08
6 months			
XD			
PF	0.57 (0.38-0.85)	0.64 (0.42-0.97)	0.03
SMF	0.02 (0.0-0.21)	0.03 (0.0-0.36)	0.005
XN			
PF	0.70 (0.50-0.98)	0.69 (0.48-1.03)	ns
SMF	0.46 (0.21-1.01)	0.34 (0.13-0.89)	0.03
1 year			
XD			
PF	0.66 (0.46-0.95)	0.66 (0.44-0.97)	0.04
SMF	0.56 (0.26-1.21)	0.72 (0.35-1.49)	ns
XN			
PF	1.00 (0.75-1.33)	0.99 (0.73-1.34)	ns
SMF	0.44 (0.21-0.91)	0.45 (0.21-0.98)	0.04

Abbreviations: MVA = multivariate regression analyses; OR = odds ratio; XD = xerostomia during daytime; XN = xerostomia at nighttime; PF = parotid gland flow (ml/ min); SMF = submandibular/ sublingual gland flow (ml/ min); ns = not significant.
* Model 1: adjusted for confounding factors (age, sex, surgery, chemotherapy and pre-RT parotid or submandibular gland flow; where appropriate).
† Model 2: final model; model 1 mutually adjusted for PF or SMF; where appropriate.
§ *p*-value for PF and SMF in model 2.

Discussion

This is the first study to report on the impact of the major salivary glands on dry mouth complaints during both day *and* night after RT for HNC. Overall, sixty-five percent of the patients still had severe complaints of dry mouth (during daytime and/ or at night; *Figure 1*) one year after RT. Submandibular gland function was shown to be the most significant determinant for dry mouth complaints during the night at all timepoints. Consequently, severe dry mouth at night was frequently observed (65% at 6 weeks to 56% at one year after RT) in this cohort of patients where no active attempt was made to spare the contralateral submandibular gland. This also appeared to affect sleeping as patients with severe dry mouth at night 12 months after RT did report significant more sleeping problems compared with patients without severe dry mouth at night (19% versus 2%).

For severe complaints of dry mouth during the day, parotid gland function was more important than submandibular gland function at 6 weeks and one year after RT. However at 6 months, the submandibular gland emerged as the strongest factor influencing severe dry mouth during daytime (XD) with a remarkable low and significant odds ratio (*Table 2*). Although this could be due to chance, it probably reflects the complex relationship between recovering secretions from the different salivary glands after RT and complaints of dry mouth during daytime. Stimulated saliva is reported to contribute as much as 80% to 90% of the average daily saliva production with varying contributions from the major salivary glands [6,15]. Remarkably, before start of treatment, the stimulated parotid gland flow rate was significantly lower compared with the submandibular gland flow rate (38% versus 62% of total output) although the latter does include sublingual saliva, secondary to the sampling technique.

An important factor linked to xerostomia could be minor salivary gland dysfunction, which is not taken into account here. The minor glands' secretion is small (7-8% of whole saliva) and difficult to measure. Radiation dose to the oral cavity (as surrogate for minor gland dose) was shown to predict the severity of xerostomia in one study [16]. However, sparing of the non-involved oral cavity with IMRT can be very

challenging due to the close proximity to the target volumes in most HNC.

Several previous studies found relatively weak associations between salivary flow and patient-reported xerostomia scores after RT [5,16-20]. The study by Eisbruch *et al.* [16] resembles this analysis to some extent. A multivariate model was used to assess which factors affected the severity of xerostomia. The authors found that none of the stimulated or unstimulated salivary flow rates from the parotid glands, submandibular glands, or combined, explained the variation in xerostomia scores. However, submandibular gland mean dose was found to be a significant explanatory variable, together with oral cavity mean dose (as mentioned before).

Several studies mentioned are hampered by relatively small patient numbers, which can make the detection of significant differences difficult, especially when the underlying data is subject to relative large variability (for example parotid and submandibular flow). Another difference with this study is the general use of xerostomia scores that are composed out of several questions related to different aspects of xerostomia (composite score). Most aspects, however, are not specific to a dry mouth caused by radiotherapy [5]. For example, swallowing problems can be caused by damage to the pharyngeal constrictors [21]. Difficulty in eating or wearing dentures may also be caused by mucositis early after RT. To evaluate the distinct impact of parotid and submandibular flow on the sensation of dry mouth, only two core questions were used in this study.

In conclusion, this study emphasizes the importance of differentiating between complaints during day- and nighttime in xerostomia research. A recently developed questionnaire specifically addresses this issue [22]. We have shown that the sensation of dry mouth at night is a frequent problem after (parotid-sparing) RT for HNC and is explained by submandibular gland dysfunction. Dry mouth during daytime is a more complex phenomenon depending on the function of the parotid gland, but also the submandibular gland is involved. Sparing the contralateral submandibular gland, in addition to parotid gland sparing, may result in improved patient-reported xerostomia and is the subject of ongoing research at our department [23].

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MUC5B levels in submandibular gland saliva of patients treated with radiotherapy for head-and-neck cancer: a pilot study

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Radiat Oncol 2012;7:91

Abstract

Introduction: The salivary mucin MUC5B, present in (sero)mucous secretions including submandibular (SMG) saliva, plays an important role in the lubrication of the oral mucosa and is thought to be related to the feeling of dry mouth. We investigated if MUC5B levels in SMG saliva could distinguish between the presence or absence of severe dry mouth complaints 12 months after radiotherapy (RT) for head-and-neck cancer (HNC).

Patients and methods: Twenty-nine HNC patients with a residual stimulated SMG secretion rate of ≥0.2 ml/ 10 min at 12 months after RT were analyzed. MUC5B (in U; normalized to 1) and total protein levels (mg/ ml) were measured in SMG saliva at baseline and 12 months after RT using ELISA and BCA protein assay, respectively.

Results: Overall, median MUC5B levels decreased after RT from 0.12 to 0.03 U ($p = 0.47$). Patients were dichotomized into none/ mild xerostomia (n = 12) and severe xerostomia (n = 17) based on a questionnaire completed at 12 months. SMG and whole saliva flow rates decreased after RT but were comparable in both groups. The median MUC5B was higher in patients with no or mild xerostomia compared to patients with severe xerostomia (0.14 vs 0.01 U, $p = 0.22$). Half of the patients with severe xerostomia had no detectable MUC5B at 12 months after RT. No differences in total protein levels were observed.

Conclusions: Qualitative saliva parameters like MUC5B need further investigation in RT-induced xerostomia. This pilot study showed a trend towards lower MUC5B levels in the SMG saliva of patients with severe xerostomia 12 months after RT for HNC.

Introduction

Xerostomia after radiotherapy (RT) for head-and-neck cancer (HNC) has a major impact on quality of life in HNC survivors [1,2]. Sparing of the parotid glands (PG) using intensity-modulated radiotherapy (IMRT) significantly improves parotid gland function in patients treated for HNC [3,4]. In some studies however, the use of parotid gland sparing RT alone did not improve patient-reported xerostomia [4,5]. Probably the submandibular, sublingual and minor salivary glands play an important role in the subjective sense of moisture in between meals [6]. They secrete glycoproteins (mucins) that cover and protect the underlying mucosa (MUC5B and MUC7). The larger salivary mucin MUC5B, present in (sero)mucous secretions including submandibular gland (SMG) saliva, is thought to be related to the perception of dry mouth by retaining moisture in the mucosa [7,8].

Our hypothesis in this pilot study was that MUC5B levels, as a qualitative parameter in human saliva, could better explain xerostomia compared with quantitative saliva measurements in RT patients. For that purpose, we investigated if MUC5B levels in SMG saliva could distinguish between the presence or absence of severe dry mouth complaints 12 months after RT for HNC.

Patients and methods

Patients

Twenty-nine patients were selected from a larger population, included in prospective studies on salivary gland function after RT for HNC at our department [9,10]. The selected patients all had a residual stimulated SMG secretion rate of ≥0.2 ml/ 10 min at 12 months after RT. The amount of 0.2 ml is the threshold for MUC5B analysis in saliva.

Saliva flow measurements

Techniques that were used for objective saliva measurements have been described previously [3,9]. Stimulated salivary flow rates were measured before treatment and at one year after RT. Citric acid solution (5%) was applied on the anterior part of the tongue every

60 seconds, for 10 minutes. Saliva near Wharton's duct orifices in the floor of the mouth was collected by gentle suction with a micropipette, representing predominantly SMG saliva but also varying amounts from the sublingual glands (SLG). Stimulated PG saliva was collected separately using Lashley cups. After collection, saliva samples were stored at -20°C until analysis.

Saliva protein assays

MUC5B levels in the SMG saliva samples were determined by an enzyme-linked immunosorbent assay (ELISA) as described previously [11,12]. The monoclonal antibody F2 used for quantification of MUC5B specifically recognizes the terminal part of the carbohydrate moiety, sulfo-Lewis^a SO_3-3Gal_1-3GlcNAc. This structure is present on MUC5B secreted by the SMGs, SLGs and palatal (minor) salivary glands. MUC5B was quantified by comparison to unstimulated whole saliva from a pooled sample of 10 healthy staff members of a dental faculty with optimal oral health. Each study patient was compared with the pooled sample of these healthy volunteers. MUC5B levels were expressed in relative units, with the MUC5B concentration in the pooled saliva of healthy volunteers normalized to 1. One unit (U) is approximately 230 µg/ ml [13].

The total protein content (mg/ ml) was measured in SMG saliva using the BCA Protein Assay Reagent (Pierce, Rockford, IL, USA) with bovine serum albumin (BSA) as a standard.

Assessment of patient-reported xerostomia

All patients completed a xerostomia questionnaire (XQ) before RT and 12 months after RT. The XQ contains questions related to xerostomia and is scored on a 5-point Likert scale. A score of '1' means no complaints, while a score of '5' implies complaints are always present. In this analysis, we utilized two questions addressing the sensation of dry mouth during daytime ('Do you have a dry mouth during the day') and nighttime ('Do you have a dry mouth at night'). Xerostomia was dichotomized into 'severe' (grade 4-5) or 'none-to-mild' (grade 1-3). Patients who had grade 4-5 xerostomia during the day and/ or the night at 12 months were grouped together. Patients with no or mild

complaints (grade 1-3) during day- and nighttime were also grouped together in the analyses.

Statistical analysis

Patient characteristics and MUC5B/ protein levels were reported using descriptive statistics (median, ranges or proportions; where appropriate). Correlations were calculated using Pearson's correlation coefficient (*r*). Paired samples obtained before and after RT were compared using Wilcoxon signed ranks test. Subgroup differences in saliva flow rate, MUC5B and protein levels were analyzed using the Mann-Whitney *U* test. Fisher's Exact test was used to compare proportions within cross tabulations. All analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). A *p*-value of <0.05 was considered statistically significant.

Results

The 29 patients included in this pilot study had a mean age of 58 years (range 35-82) and 22 (76%) were male. All patients had a squamous cell carcinoma of the head and neck, with 19 oropharyngeal tumors (66%), 6 laryngeal (21%), 3 nasopharynx (10%) and 1 oral cavity tumor (3%). IMRT was used in 21 patients (72%), the remainder was treated with conventional (2D) techniques [9]. The mean dose to the SMGs was 55.9 Gy (range 10.6-71.1 Gy).

Overall, the median MUC5B level decreased after RT from 0.12 U to 0.03 U (p = 0.47). MUC5B levels at 12 months showed a very weak correlation with the mean SMG dose in Gy (r = 0.18). The median total protein content of SMG saliva decreased slightly after treatment (1.00 and 0.82 mg/ ml respectively, p = 0.62).

Twelve months after RT, 17 patients had severe complaints of dry mouth at day- and/ or nighttime, 12 patients had no or mild complaints. The SMG and whole saliva (PG + SMG) flow rates decreased after RT but were comparable in both groups at 12 months (*Table 1*).

No statistical differences were found in the baseline MUC5B levels between the groups (median 0.12 versus 0.14 U for the group with and without severe xerostomia, respectively) nor in the change from

Table 1 Median saliva flow rates, MUC5B and total protein levels 12 months after RT in patients with and without severe xerostomia during daytime and/ or nighttime.

		No/mild xerostomia *(n= 12)*	**Severe xerostomia** *(n= 17)*	***p***
SMG flow rate	ml/ 10 min	0.69	0.80	0.66
WS flow rate (PG + SMG)	ml/ 10 min	3.35	2.80	0.82
MUC5B concentration	U	0.14	0.01	0.22
Undetectable MUC5B *	%	25	47	0.27
MUC5B x flow rate †	µg/ 10 min	15.0	1.3	0.37
Total protein	mg/ ml	0.87	0.82	0.76

Abbreviations: SMG = submandibular gland, WS = whole saliva, PG = parotid gland, U = units; normalized to 1 for unstimulated saliva in healthy controls.
***** MUC5B level in SMG saliva equal to 0.00 U at 12 months after RT.
† The MUC5B x flow rate product (in µg/ 10 min) was calculated by multiplying the MUC5B concentration (in U) and SMG flow rate (in ml/ 10 min) for each patient at 12 months after RT and assuming 1 Unit is 230 µg/ ml of MUC5B [13].

baseline in each individual patient (calculated as ΔMUC5B: median 0.04 versus 0.11 U respectively, p = 0.9). ΔMUC5B showed a small negative correlation with the SMG mean dose in Gy (r = -0.26).

At 12 months, the median MUC5B was higher in patients with no or mild xerostomia compared to patients with severe complaints, although the difference was not statistically different at the 0.05 level (*Table 1*). The group with severe complaints was characterized mainly by undetectable MUC5B levels and a number of outliers (*Figure 1*). Two of the outliers represented a 14- and 123-fold increase in the MUC5B concentration from baseline respectivaly. Repeating the analyses without these two extremes showed a borderline significant higher MUC5B level in the patients with no or mild xerostomia (median 0.14 vs 0.00 U, p = 0.055) at 12 months.

When we combined the qualitative (MUC5B in U) and quantitative (SMG flow rate in ml/ 10 min) measurements for each individual patient by multiplying both parameters (MUC5B x flow rate; *Table 1*) and assumed 1 unit is 230 µg/ ml of MUC5B [13], we found an approximate 10-fold lower value (in µg/ 10 min) in the group of patients with severe complaints of dry mouth, although statistical difference was not reached in this small study.

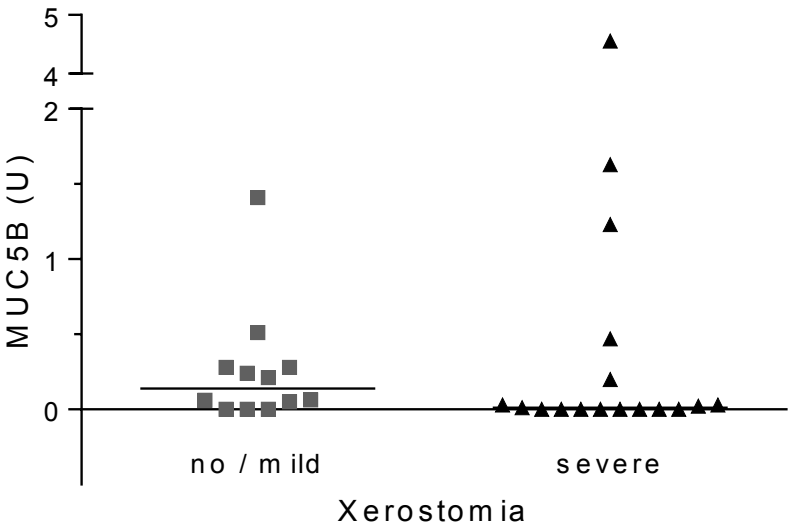

Figure 1 Comparison of MUC5B levels (in U) in patients with and without severe xerostomia 12 months after RT, measured using ELISA. The horizontal line represents the median for each group.

Discussion

This pilot study did not show a statistically significant difference in MUC5B levels in SMG saliva of patients with and without severe xerostomia 12 months after RT, although a trend was observed towards higher MUC5B levels in patients with fewer complaints of dry mouth. Almost half of the patients with severe xerostomia had no detectable MUC5B at 12 months after RT. The results are therefore of interest and do need investigation within a larger cohort of patients. An ongoing prospective study at our department, investigating the effect of sparing the contralateral SMG on xerostomia after RT, is expected to yield more SMG saliva samples for future qualitative analyses [10].
As both subgroups in this study had comparable amounts of saliva but differed in the severity of their complaints, a case is made for qualitative saliva parameters rather than quantitative measurements in xerostomia research. The high-molecular weight salivary mucin MUC5B contains large carbohydrate groups that are heterogeneous and include sulfated and sialylated oligosaccharides, retaining large

amounts of water. The unique rheological properties of MUC5B contribute to the formation of a thin salivary film and the resulting coating is thought to hydrate and lubricate the soft tissues of the mouth [14]. Serous acinar cells found in the parotid glands do not produce mucins. The latter may explain why, in RT for head-and-neck cancer, sparing of the parotid glands alone does not seem to improve patient-reported xerostomia [4].

Apart from the free MUC5B fraction measured in SMG saliva in this study, there may be other mucin-related factors that can explain xerostomia. First, in stead of the free fraction, the amount of mucosa-bound MUC5B may better explain which patients will complain of a dry mouth. Pramanik *et al*. showed, that in (non-RT) dry mouth patients unable to provide a measurable unstimulated saliva sample (zero flow), MUC5B was often still present on all mucosal surfaces [15]. Therefore, mucins retained on the mucosa of dry mouth patients are presumably less hydrated than in normal subjects. In this regard, post-translational modifications of MUC5B synthesis and in particular sulfation levels (rather than mucin levels per se) could result in a reduced water content of mucins and explain the dry mouth sensation. Loss of MUC5B sulfation was observed in the mucous acini from labial salivary glands of patients with Sjögren syndrome and was unrelated to alterations in saliva quantity [7]. To what extent these findings can be extrapolated to patients with radiation-induced xerostomia needs to be investigated. Third, differently glycosylated MUC5B species are present in saliva. In single glandular secretions and even in one secretory acinus different glycoforms are expressed, pointing to a large heterogeneity in mucin molecules [16]. Moreover, MUC5B from different glandular sources have different rheological properties that may influence fluid retention on mucosal surfaces [17]. In this study, a specific (sulfo)glycolysation motif (sulfo-Lewis^{a}) was detected, present on MUC5B secreted by the SMGs, SLGs and palatal minor glands. Possibly, (absence of) other MUC5B glycoforms can better explain why some patients with recovering SMG secretion after RT complain of a dry mouth and others do not.

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KORA

6 Submandibular gland dose-response relationships after radiotherapy for head-and-neck cancer

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Submitted

Abstract

Purpose: To construct a normal tissue complication probability (NTCP) curve for submandibular gland (SMG) function after radiotherapy (RT) based on mean dose and selective flow measurements.

Patients and methods: We utilized dose-response data of 151 head-and-neck cancer patients included in prospective salivary gland function studies between 1999 and July 2012. SMG flow rates were measured 6 weeks and 1 year post-RT and converted into the percentage of baseline. SMG-data were fitted to the Lyman-Kutcher-Burman (LKB) model with a complication defined as SMG flow ratio <25% of the pre-RT flow rate (RTOG/ EORTC grade 4 xerostomia).

Results: In general, a decrease of SMG function with increasing mean dose was observed. LKB-modelling showed substantial shift of the NTCP-curve between 6 weeks and 1 year post-RT: the TD_{50} (mean dose leading to 50% NTCP) was 22.7 Gy and 35.0 Gy, respectively. The curves intersected at 40 Gy mean dose. Of the cSMGs that received a mean dose above 40 Gy, 33% (38/114 glands) showed recovery of the flow ratio to ≥25% of baseline at 6 weeks, compared with 14 out of 78 glands (18%) at 1 year after RT (p = 0.021). Below 40 Gy, 59% (19/32 glands) versus 77% (17/22 glands) recovered to the same level at 6 weeks and 1 year after RT, respectively (p = 0.24).

Conclusions: We constructed a NTCP-curve of SMG function after radiotherapy to aid treatment planning in head-and-neck cancer. Above 40 Gy mean SMG dose, NTCP worsened between 6 weeks and 1 year post-RT. The TD_{50} at 1 year was equal to 35 Gy, suggesting similar radiosensitivity (in terms of volumetric output) of the SMG as compared with the parotid gland late after RT.

Introduction

Although sparing of the parotid glands (PG) is feasible with intensity-modulated radiotherapy (IMRT) for head-and-neck cancer (HNC), gains in patient-reported xerostomia have been absent or relatively small [1-3]. Radiation-induced xerostomia has a very significant impact on the quality of life of HNC survivors [4,5]. Because of the functional characteristics of the submandibular gland (SMG) and the mucins contained in its saliva, this gland appears important with respect to the subjective sense of moisture [6,7]. The rate of patient-reported xerostomia after RT has been shown to decrease following additional sparing of SMG function [8-10]. Because the ipsilateral SMG inevitably receives high radiation doses due to its close proximity to the tumor in most HNC, advanced IMRT-techniques are aimed at sparing the contralateral SMG (cSMG) [11-12]. Knowledge of dose-response relationships for the SMG is crucial to aid treatment planning in HNC patients. To date, only one paper has been published regarding continuous dose-response relationships for the SMG based on selective measurements [13]. In this study, we constructed a normal tissue complication probability (NTCP) curve of SMG function after RT based on selective flow measurements performed in a large cohort of HNC patients.

Patients and methods

For the description of submandibular gland NTCP, we utilized the dose-response data of 151 patients with various HNC (TNM stages T1-4N3M0; TNM-classification, 7th edition, 2010) treated at our department. Their SMG function was measured in prospective salivary gland function studies performed between 1999 and July 2012 [14-16]. The diagnosis was histologically confirmed in all patients. No previous radiotherapy of the salivary glands was allowed nor did any patient have a malignancy or other disease of the salivary glands. All studies described were approved by the Medical Ethics Committee of the University Medical Center Utrecht. Informed consent was obtained from each patient.

Radiotherapy

Details on treatment planning and delineation have been published previously [14-16]. Twenty-nine patients received conventional radiotherapy (CRT) using mainly opposing lateral photon beams. Seventy-eight patients were treated with parotid gland sparing IMRT aimed at sparing both PGs. Recently, we implemented an optimized IMRT-technique with the intention to spare both PGs and the cSMG [11]. Forty-four patients with oropharyngeal cancer were included in a prospective analysis of this technique. Patients with contralateral nodal disease (*N*-stage N2c) were specifically excluded from the latter study.

The prescribed dose to the tumor GTV was generally 69-70 Gy in 30-35 daily fractions, depending on the use of concomitant chemotherapy in case of locally advanced disease (T-stages T3-T4). Consequently, the elective lymph node regions were prescribed either 54 Gy in 1.8 Gy daily fractions (integrated boost) or 46 Gy in 2 Gy fractions (conventional fractionation). Indications for elective treatment of the contralateral level II-IV lymph node regions were N2a-N2b nodal stage, a tumor crossing the midline or a base of tongue carcinoma. Patients were treated 5 times per week.

Gross tumor volume (GTV) delineation was performed on contrast-enhanced CT images with 3 mm slice thickness (registered to MRI) in the treatment position with an immobilization mask. Organs at risk (spinal cord, brain, PGs and SMGs) were delineated on every CT slice together with the target volumes. When available, MRI sialography aided in the delineation of the SMGs, especially in the cranial part of the gland [17]. If not already performed, the SMGs were outlined for the purpose of this study and mean doses were calculated using the 3D-dose distributions from archived treatment plans.

Parotid and submandibular flow measurements

Techniques that were used for selective saliva measurements have been described previously [15,16]. To avoid the influence of diurnal variation in salivary flow, consecutive measurements were scheduled as much as possible at the same daytime for each patient. Patients were instructed not to eat or drink 60 minutes before saliva collection. The collected saliva samples were weighted and converted to ml/ min, assuming a specific gravity of 1.0 g/ ml.

Stimulated salivary flow rates were measured before treatment, at 6 weeks and 1 year after RT. Citric acid solution (5%) was applied on the mobile part of the tongue every 60 seconds, for 10 minutes. PG saliva was selectively collected using Lashley cups. Saliva near Wharton's duct orifices in the floor of the mouth was collected by gentle suction with a micropipette. This represents predominantly SMG saliva but also small secretions from the sublingual glands (SLGs), which exit nearby or through the same orifice. Because of the close proximity of both duct orifices, only the combined output of the bilateral SMGs could be measured. As a result of this, the pre-RT SMG flow rate was halved to represent the output per gland unless the patient underwent an ipsilateral neck dissection pre-RT (including the ipsilateral SMG). At 6 weeks and 1 year after RT, all measured SMG saliva was assumed to be produced by the cSMG. The mean dose to the ipsilateral SMG was on average 65 Gy and higher than 40 Gy in all cases. Above 40 Gy, no significant recovery of SMG function was observed after RT in the study by Murdoch-Kinch *et al.* [13].
The cSMG flow rate at 6 weeks and 1 year after RT was converted into the percentage of baseline (unilateral) SMG flow rate. For NTCP-modelling, a cSMG complication was defined as cSMG flow ratio <25% of the pre-RT flow rate, or grade 4 xerostomia according to the Radiation Therapy Oncology Group/ European Organization for Research and Treatment of Cancer (RTOG/ EORTC) Late Effects Consensus Conference [18]. In addition, if the cSMG flow rate at 6 weeks or 1 year post-RT exceeded 0.55 ml/ 10 min (equal to 25% of the cohort's mean baseline unilateral SMG flow rate of 2.2 ml/ 10 min; range 0.10-9.84) irrespective of a flow ratio <25%, this was considered as 'no complication' present. This exception was made in order to correct for large interpatient variation and extreme positive outliers in the baseline (pre-RT) SMG flow rates.

Normal tissue complication probability model
The cSMG mean dose was used for NTCP-modelling. The dichotomized complication data were fitted to the NTCP-model proposed by Lyman, Kutcher and Burman (LKB-model) [19] using maximum likelihood optimization as described previously [20,21]. This model is assumed to quantitatively establish the effects of both radiation dose and

irradiated volume on the probability of radiation-induced changes in salivary gland function. Three parameters are present in the sigmoid dose-response relationship described in the LKB-model. TD_{50} is the dose at which a 50% complication probability is seen after uniform salivary gland irradiation and parameter *m* describes the slope of the NTCP-curve. Parameter *n* accounts for the volume effect of an organ and depends on the tissue organization. Because we wanted to model the SMG function at 6 weeks and 1 year after RT as function of the mean dose, we fixed the value of *n* at 1. To compute the confidence intervals (CI) for TD_{50} and *m*, 10.000 pseudo-datasets were generated with a Monte Carlo method and subsequently fitted to the LKB-model [22]. The CI was derived directly from the fitted parameter-set.

Statistical analysis

Baseline patient characteristics were reported using descriptive statistics (mean, ranges or proportions; where appropriate). Fisher's Exact test was used to compare proportions within cross tabulations. Patients that were lost to follow-up, missed their appointment or had recurrent illness were blinded in the analysis. The NTCP-models were constructed using Matlab® release 2011a (MathWorks, Natick, MA, USA). Statistical analyses were performed using SPSS Statistics version 20 (IBM Corporation, Armonk, NY, USA). A *p*-value of <0.05 was considered statistically significant.

Results

Baseline patient, tumor and treatment characteristics are shown in *Table 1*.

Figure 1 shows the NTCP-curves at 6 weeks and 1 year post-RT after fitting the cSMG flow data to the mean dose LKB-model (*n* fixed at 1). At 6 weeks after RT (146 endpoints available), the NTCP-curve shows a plateau around 65-70% at mean doses higher than 60 Gy. The TD_{50} was 22.7 Gy (95% CI: 11.7-37.1 Gy) and *m* equaled 1.57 (95% CI: 0.87-3.14). At 1 year (100 endpoints available), SMG function showed a gradual decrease with increasing mean dose (*m* = 0.44, 95% CI: 0.28-0.65). The NTCP-curve shifted to the right, reflected in the TD_{50} of 35.0 Gy (95% CI: 27.8-41.5 Gy). The curves intersect at 40 Gy mean dose. Of the cSMGs that received a mean dose above 40 Gy, 33% (38/114 glands) showed recovery of

Table 1 Patient, tumor and treatment characteristics, n (%).

Gender	
Male	100 (66)
Female	51 (34)
Age (mean; range)	60 (35-84)
Tumor site	
Larynx	16 (11)
Hypopharynx	5 (3)
Oropharynx	106 (70)
Nasopharynx	18 (12)
Oral cavity	4 (3)
Unknown primary	2 (1)
T-stage	
T1	26 (17)
T2	78 (52)
T3	28 (19)
T4	17 (11)
Tx	2 (1)
N-stage	
N0	50 (33)
N1	30 (20)
N2a-b	55 (36)
N2c	10 (7)
N2 nasopharynx	4 (3)
Nx	2 (1)
RT modality	
IMRT	122 (81)
CRT	29 (19)
Mean dose (Gy; range)	
PG	34.7 (3.1-71.4)
iSMG	65.1 (43.6-72.6)
cSMG	50.8 (7.0-72.1)
Surgery	
Tumor ± iND	13 (8)
iND only	16 (11)
None	124 (81)
Chemotherapy	30 (20)

Abbreviations: IMRT = intensity-modulated radiotherapy; CRT = conventional radiotherapy; PG = parotid gland; iSMG = ipsilateral submandibular gland; cSMG = contralateral submandibular gland; iND = ipsilateral neck dissection (including the ipsilateral SMG).

the flow ratio to ≥25% of baseline at 6 weeks, compared with 14 out of 78 glands (18%) at 1 year after RT (p = 0.021). Below 40 Gy, 59% (19/32 glands) and 77% (17/22 glands) recovered to the same level at 6 weeks and 1 year after RT, respectively (p = 0.24).

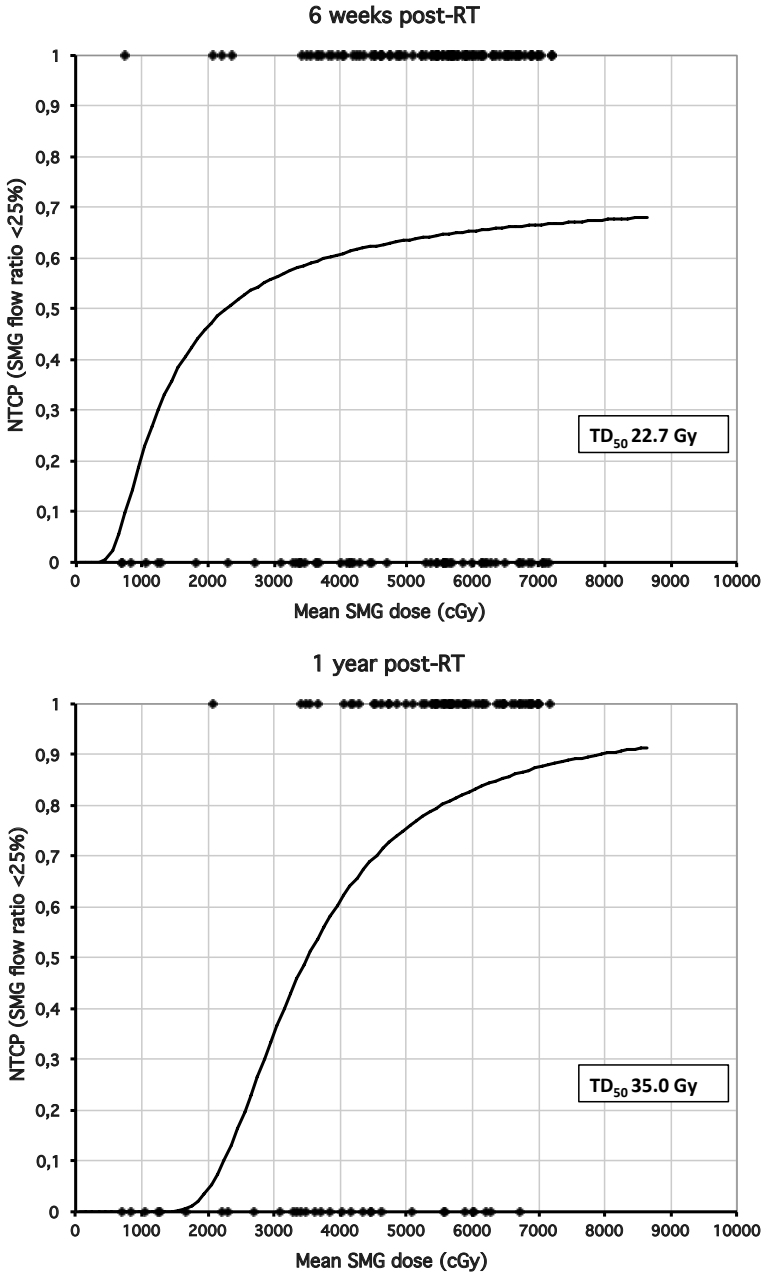

Figure 1. NTCP-curves as a function of SMG mean dose at 6 weeks and 1 year post-RT (LKB-model). Clinical endpoints are plotted as diamonds.
Abbreviations: NTCP = normal tissue complication probability; LKB-model = Lyman-Kutcher-Burman model using the mean dose; SMG = submandibular gland; TD_{50} = (mean) dose at which 50% complication probability is observed.

Discussion

This study reports the dose-response relationships for the submandibular gland after radiotherapy based on mean dose and selective flow measurements in a large cohort of HNC patients. The NTCP-curve and TD_{50} value at 1 year reported here are similar to the plot for stimulated submandibular saliva toxicity published previously [13]. Between 6 weeks and 1 year after RT, the NTCP-curve showed substantial shift to the right (TD_{50} of 23 and 35 Gy, respectively) reflecting a tendency towards recovery of function for the same mean dose considered. Using multivariate modelling, Murdoch-Kinch *et al.* found a threshold dose of 39 Gy, above which no recovery of (un)stimulated SMG function was seen during a follow-up period of 24 months after RT. Our dose-response curves did not reveal such a threshold dose. However, SMGs that received mean doses over 40 Gy had (on average) only a 18% chance of output recovery to ≥25% of baseline at 1 year after RT. Differences in this respect probably stem from different models and endpoints used, or the distribution of the underlying data. The results suggest a comparable radiosensitivity in terms of selective flow measurements for the parotid gland (PG) and SMG at 1 year after RT. Previously published NTCP-data for the PG showed a TD_{50} of 40 Gy (95% CI: 37.3-42.8 Gy) at 1 year [23]. The limited clinical data available on selective flow measurements support an equal functional loss of the PG and SMG both early and late after RT [24,25]. Observations in rats showed a slightly higher radiosensitivity of the SMG for late damage [26].

Interestingly, at 6 weeks, we found a TD_{50} for SMG function (23 Gy) comparable to that for PG function after IMRT (26 Gy; IMRT was used in 81% of the patients in this study) [20]. Most notably, unlike the parotid gland, the 6 week NTCP-curve showed a plateau around 65-70% at mean SMG doses higher than 50-60 Gy and did not approach 100%, as might be expected. This is due to a relatively large number of SMGs that retained flow at above 25% of baseline in this mean dose range. At 1 year, most of these glands eventually did show a functional decline below 25% of baseline (*Figure 1*, clinical data on the x-axis). For mean

SMG doses above 40 Gy (the intersection of the NTCP-curve at 6 weeks and 1 year), NTCP worsened between 6 weeks and 1 year after RT. This could be a true effect, indicating that the SMG can show delayed functional decline after irradiation at higher mean doses (>40 Gy).
In this regard, bias could have been introduced by the assumption that all collected SMG saliva after RT was produced by the cSMG. Also, measurement errors can occur when PG saliva leaks from the Lashley cups and flows into the floor of the mouth where it is collected as SMG saliva. The latter is expected to occur at random, however. Finally, the TD_{50} at 6 weeks could be influenced by relatively few clinical data in the lower cSMG mean dose range.
Reducing the cSMG mean dose below 40 Gy is challenging but could be achieved in about 50% of HNC patients in planning studies [11,13]. In practice, it will be most feasible in patients with smaller tumors not crossing the midline and in those without the need for elective irradiation of the contralateral neck. Because the 30-40 Gy mean dose range is on the steepest part of the NTCP-curve at 1 year, even a small mean dose reduction of a few Gy in this range will translate into relatively large reductions in NTCP after RT. Every effort to reduce the cSMG mean dose below 40 Gy should therefore be undertaken, if this is safe from an oncological point of view. The results of a prospective study of cSMG-sparing on patient-reported xerostomia will be published separately.
Previous modelling studies showed that for the parotid gland (PG), mean dose models are preferred for describing dose-response relationships [21]. Similar to Murdoch-Kinch *et al.*, we assumed that the SMG resembles the PG in this respect [13]. However, clear region-dependent radiosensitivity was found for the rat parotid gland related to the presence of blood vessels, ducts [27,28] and thereby also stem cells [29] in the high-dose radiation field. If this is also the case in human salivary glands, spatial dose distributions can become more important than the mean dose concept. Further research is needed to elucidate this.

In conclusion, a mean dose NTCP-curve of post-RT SMG function was constructed to aid treatment planning in head-and-neck cancer. Above 40 Gy mean SMG dose, NTCP worsened between 6 weeks and 1 year post-RT. The TD_{50} at 1 year after RT was equal to 35 Gy, suggesting similar radiosensitivity of the SMG compared with the PG late after RT.

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KORA

7 Sparing the contralateral submandibular gland in oropharyngeal cancer patients: a planning study

Adapted from:
Houweling AC, Dijkema T, Roesink JM, Terhaard CHJ and Raaijmakers CPJ.

Radiother Oncol 2008;89:64-70

Abstract

Purpose: The submandibular glands are proposed to be important in preventing xerostomia in head-and-neck cancer patients after radiotherapy. We investigated the feasibility of sparing the contralateral submandibular gland (cSMG) by reducing the dose to the contralateral planning target volume (PTV) and by reducing the clinical target volume (CTV)-to-PTV margin.

Patients and methods: Ten oropharyngeal cancer patients that received irradiation of the contralateral elective PTV were included in this planning study, using intensity-modulated radiotherapy (IMRT). The effect of reducing the dose coverage to the contralateral elective PTV from 95 to 90% of the prescribed dose (54 Gy in 1.8 Gy daily fractions) on the mean dose to the cSMG was determined. The influence of reducing the margin for position uncertainty from 5 to 2 mm was also investigated.

Results: The mean dose to the cSMG was reduced from 54 Gy to approximately 40 Gy if the dose coverage to the contralateral elective PTV was reduced to 90% of the prescribed dose. The estimated normal tissue complication probability (NTCP) was reduced below 50%. Reducing the uncertainty margin from 5 to 2 mm resulted in a decrease of the mean dose to the cSMG of approximately 6 Gy.

Conclusions: Reducing the mean dose to the cSMG below 40 Gy is possible with an acceptable dose coverage of the contralateral elective PTV.

Introduction

Xerostomia is a severe side effect of radiotherapy (RT) in head-and-neck cancer patients. It causes difficulty in swallowing, eating, speaking and provokes an early onset of dental caries [1,2]. A large effort has been put in reducing the dose to the parotid glands [3,4]. The reduction of the dose to the parotid glands leads to a preservation of salivary flow after RT. Unfortunately, patients still complain of a dry mouth [5,6]. Radiation damage to the submandibular glands has been proposed to explain the remaining subjective feeling of a dry mouth [7-10].

Sparing the submandibular glands is, however, much more challenging than parotid gland sparing. The submandibular gland is located adjacent to the elective nodal clinical target volumes (CTVs). Due to uncertainties in target delineation and patient positioning during treatment, a margin around the CTV is applied to obtain the planning target volume (PTV). This causes an overlap between the submandibular gland and the elective nodal PTV, making its sparing complicated without compromising the dose to that PTV. The overlap between the submandibular gland and PTVs at the contralateral side of the neck is generally smaller than at the ipsilateral side, particularly for tumors not crossing the midline. Furthermore, the probability of microscopic disease is lower at the contralateral side [11]. This suggests a possibility to reduce the dose to the contralateral submandibular gland (cSMG) with an acceptable reduction of the tumor control probability (TCP) in the contralateral elective PTV [12].

Whilst there have been a few papers looking at the clinical aspects of sparing the submandibular glands [10,13,14], little has been published on the dosimetric details of such sparing. The goal of this planning study was to determine the possibility to reduce the mean dose to the cSMG and to investigate what compromises have to be made to achieve a substantial sparing. Therefore, several RT plans were made for ten oropharyngeal cancer patients. The reduction of the mean cSMG dose was determined in case of normal coverage of the PTVs and in case of a slight and local underdosage in the contralateral electively treated PTV.

Patients and methods

Patients

Ten oropharyngeal cancer patients were included in the planning study, treated with primary RT at our department between August 2003 and November 2006. Staging was performed according to the TNM staging system [15]. Indications for elective treatment were N2b nodal stage, a tumor crossing the midline or a base of tongue carcinoma. The patients had disease stages T1-3N0-2bM0 and in all cases the contralateral lymph nodes were electively treated (*Table 2*). The distribution of TNM stages in this cohort was representative for oropharyngeal cancer patients treated at our department [16].

Imaging and delineation

Each patient underwent a computed tomography (CT) scan (CT aura, Philips Medical Systems, Best, The Netherlands) with intravenous contrast. The slice thickness was 3 mm, patients were positioned with an immobilization mask. Delineation of the CTVs and the organs at risk (OARs) was performed by a radiation oncologist as described previously [17]. The contralateral elective CTV generally consists of the level II, III and IV lymph node areas as described by Gregoire *et al*. [18]. The level II lymph node area can be subdivided in level IIa and IIb. The level IIa area is located adjacent (posterior) to the submandibular gland. Sparing the cSMG can influence the dose to the contralateral electively treated CTV, in particular the level IIa lymph node area. Therefore, the dose to the complete contralateral elective CTV and the level IIa lymph node area alone should be monitored strictly, which required separate delineation of this contralateral level IIa lymph node area. The lowest dose to 1 cc of the elective PTV volume was used to monitor the dose coverage to the elective CTV.

Margins

Margins are applied to the CTVs in order to obtain the PTVs. Clinically, a margin of 5 mm is used. This margin might be decreased by applying modern position verification tools such as daily cone-beam CT or fiducial marker implantation [19]. As previously described by Van Asselen *et al*.

[20], a decrease in margin will lead to a substantial reduction in dose to the parotid glands of approximately 1.3 Gy/ mm. Margins of 5 mm and 2 mm were used here, to investigate to what extent the increased workload associated with improved position verification will result in a benefit for the submandibular glands.

Treatment planning

The planning CT scan was transported to the inverse treatment planning module of the planning system (PLATO-ITP, Nucletron, Veenendaal, The Netherlands). An intensity-modulated radiation therapy (IMRT) technique with nine equally distributed 6 MV photon beams starting at 0° was used. The plans were calculated using a dose grid of 1.9 mm. The prescribed dose was 69 Gy in 2.3 Gy daily fractions to the gross tumor volume (PTV-GTV) and 66 Gy in 2.2 Gy daily fractions to the PTV-CTV. The prescribed dose to the electively treated PTVs was 54 Gy in 1.8 Gy daily fractions, according to the clinical protocol [17].

In order to achieve salivary gland sparing and an appropriate target coverage, the parotid glands and the cSMG were divided in two parts. This was done using Volumetool, a delineation program developed at our department [21]. The border of the two parts was located at 15 mm from all PTVs in all three dimensions (*Figure 1*), which is the distance where the dose gradient can theoretically be decreased from 54 to 0 Gy. This resulted in a part adjacent to or overlapping with the PTVs and a part at a distance of 15 mm from the target volumes. Thereby, the distant part could be spared independently without influencing the dose to the PTVs. Although this distant part has a small volume, it influences the dose optimization process due to the fact that the cost function of the PLATO planning system does not take the volume size of an OAR into account.

For each patient, four different IMRT plans were made, of which the characteristics were summarized in *Table 1*. The parotid glands were spared in all plans to the minimally possible mean dose at which a target coverage of at least 95% of the prescribed dose to 99% of the volume of all the PTVs was reached. The first plan (plan A) included only the sparing of the parotid glands, while both submandibular

glands were ignored. In the second plan (plan B), the dose to 99% of the volume of the PTVs was at least 95% of the prescribed dose, which is the clinically accepted target coverage. In the third plan (plan C), a concession was allowed in the coverage of 99% of the volume of the contralateral elective PTV with the aim of sparing the cSMG. This coverage had to be at least 90% of the prescribed dose, instead of 95%. The fourth plan (plan D) was made to determine which compromises had to be made to the dose coverage of all the PTVs in order to achieve a cSMG mean dose below 40 Gy. At this dose, the normal tissue complication probability (NTCP) of the parotid gland is 50% [22]. Plan D was not generated if the mean dose to the cSMG in plan B or C was already below 40 Gy.

NTCP

The effect of a reduction of the cSMG mean dose can be expressed as a change in the NTCP. Limited research has been performed on sparing the submandibular glands [10,13,14]. Murdoch-Kinch *et al*. [13] found a threshold dose of 39 Gy, above which the submandibular flow rate was negligible after RT. This corresponds to the parotid gland dose at which 50% NTCP was observed by Roesink *et al*. [22], who defined a complication as a stimulated parotid gland flow ratio of less than 25% of the pre-RT flow rate (or grade 4 xerostomia according to the Radiation Therapy Oncology Group/ European Organization for Research and Treatment of Cancer (RTOG/ EORTC) consensus) [23]. This NTCP-curve was applied to the submandibular gland in this planning study.

Results

Dose distributions

In general, dose distributions were obtained that met our criteria with regard to dose homogeneity and OAR sparing (*Figure 1*). The volume that received more than 107% of the prescribed dose was always a small volume located inside the GTV and the dose to the target volumes was within the constraints described in *Table 1*. The dose to the brain and spinal cord was always within the clinical constraints.

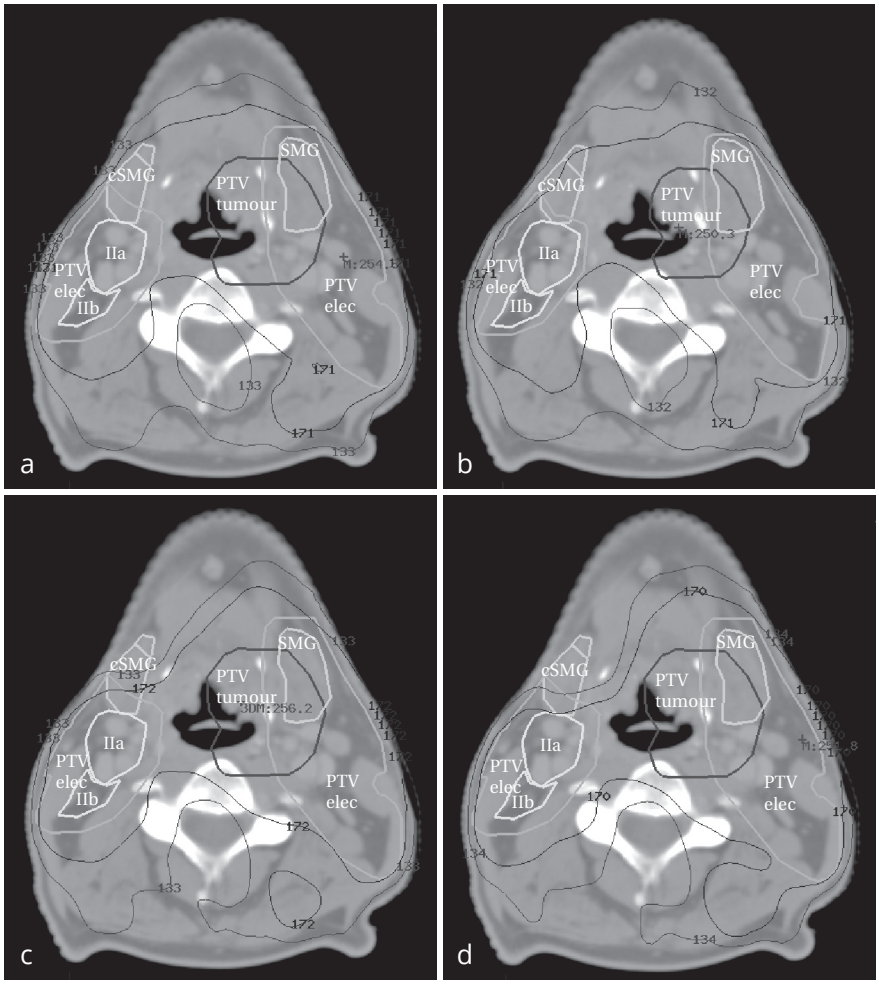

Figure 1. Typical dose distributions for the different IMRT plans aiming at sparing the cSMG in one oropharyngeal patient. The margin to obtain the PTV was 5 mm in a, c and d; in b it was 2 mm. In a and b (plan A), only the parotid glands were spared. The cSMG was spared with a dose to the contralateral electively treated PTV of at least 95% in c (plan B) and this dose was reduced to 90% in d (plan C).
The thick lines are the delineated volumes: the PTV of the tumor (PTV tumor), the electively treated PTVs (PTVelec), level IIa and IIb of the contralateral elective CTV (IIa and IIb) and the SMG. The cSMG is divided in a part adjacent to or overlapping with the PTVs and a part at a distance of 15 mm from the target volumes. The thin lines are the isodose lines. The 171 cGy isodose line is 95% of the prescribed dose to the elective volumes; the 133 cGy line is the 40 Gy isodose line.

Table 1 The characteristics of the different IMRT plans performed in this planning study.

Plan	Constraints
A	All PTVs: >95% of the prescribed dose
	No cSMG sparin
B	All PTVs: >95% of the prescribed dose
C	Contralateral elective PTV: >90% of the prescribed dose
	Other PTVs: >95% of the prescribed dose
D	Mean dose to the cSMG <40 Gy

When the cSMG was spared more rigorously, the dose gradient between the contralateral electively treated PTV and the cSMG became steeper. This resulted in a movement of, for example, the 40 Gy and 51 Gy isodose lines (*Figure 1*).

Mean dose and NTCP

The average of the mean dose to the cSMG for the ten patients in plan A, where no cSMG sparing was performed, was approximately 57 Gy for both the margins (*Figure 2a*), which resulted in an average NTCP value of approximately 85% (*Figure 2b*). The average of the minimal dose to the contralateral elective PTV was sufficient for both margins, approximately 50 Gy (*Figure 2a and Table 3*).

The efforts in sparing the cSMG without concessions to the dose coverage of the target volumes (plan B) resulted in an average of the mean dose to the cSMG of 54 Gy for the 5 mm margin plans. The average of the mean dose values for the 2 mm margin plans was 49 Gy. The corresponding NTCP values were 80% and 70%, respectively. The average of the minimal dose to the contralateral elective PTV did not decrease (*Figure 2a* and *Table 3*).

The reduction in the dose coverage of the contralateral elective PTV from 95% to 90% of the prescribed dose (plan C) resulted in an average of the mean dose to the cSMG of approximately 40 Gy for both margin plans. The average of the minimal dose to the contralateral elective PTV decreased to 48 Gy for both margin plans (*Figure 2a*). One plan was excluded in the averaging for both margins, due to an underdosage of the primary PTV (*Table 2*).

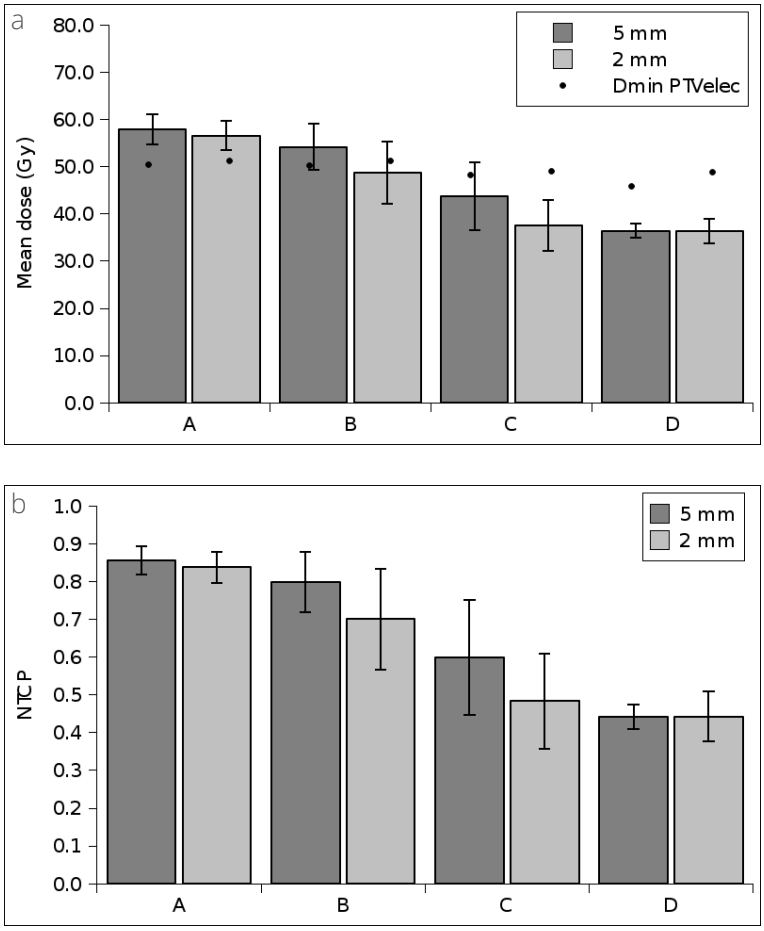

Figure 2 The average (SD) of the mean doses (a) and NTCP values (b) of the IMRT plans aiming at cSMG sparing in oropharyngeal cancer patients. On the horizontal axis, the plan labels are denoted. In plan A only the parotid glands were spared. The cSMG was spared with a dose coverage to the contralateral elective PTV of 95% in plan B and 90% in plan C. In plan D, the aim was to reduce the mean cSMG dose below 40 Gy.
One plan was excluded in the average of plan C for both margins. In the average of plan D, respectively, four and two plans were excluded for the 5 mm and 2 mm margin plans. The error bars denote the standard deviation of the plans. The dots represent the average of the minimal dose of the contralateral elective PTV (a).

The average of the mean cSMG dose values in plan D was 36 Gy for both margins. The average of the minimal dose to the contralateral elective PTV decreased to 46 Gy in the 5 mm plans, but remained constant for the 2 mm margin plans. Four 5 mm margin plans were

Table 2 Dose parameters for submandibular gland sparing for all patients.

TNM-stage	Plan C				Plan D			
	PTV_{prim}		PTV_{elec}	cSMG	PTV_{prim}		PTV_{elec}	cSMG
	D_{min} (Gy)	$V_{<95\%}$ (%)	D_{min} (Gy)	D_{mean} (Gy)	D_{min} (Gy)	$V_{<95\%}$ (%)	D_{min} (Gy)	D_{mean} (Gy)
T2 N0	62.5	1.3	49.9	37.74	62.5	1.3	49.9	37.74
T2 N1	64.1	0.2	45.6	34.08	64.1	0.2	45.6	34.08
T2 N2b	64.3	1.0	47.7	53.38	54.6*	2.6*	40.1*	38.82*
T1 N2b	62.3	0.9	48.4	50.63	55.8*	2.5*	44.0*	34.47*
T1 N2b	62.9	0.7	47.3	44.84	64.1	0.4	41.8	36.44
T2 N0	62.9	0.5	48.1	35.29	62.9	0.5	48.1	35.29
T3 N0	60.4*	1.9*	48.8*	43.15*	59.5*	1.6*	48.1*	40.06*
T2 N2b	62.9	0.6	47.9	41.10	62.1	1.0	45.2	37.84
T3 N0	60.3†	1.5	50.0	51.50	55.3*	4.3*	49.4*	46.88*
T1 N0	62.1	1.5	48.9	45.03	62.6	0.8	43.6	37.22

Abbreviations: PTVprim = primary PTV; PTVelec = contralateral elective PTV; Dmin = minimal dose (Gy) to 1 cc of the volume; V<95% = volume (%) that received <95% of the prescribed dose; Dmean = mean dose (Gy).
* Plan excluded based on PTV-CTV underdosage as a result of cSMG sparing.
† Coverage of the PTV-CTV in this plan is the same as in plan A.

Table 3 Average dose parameters for submandibular gland sparing using different planning strategies.

	PTV_{prim}			PTV_{elec}			cSMG
	D_{min} (Gy)	D_{mean} (Gy)	$V_{<95\%}$ (%)	D_{min} (Gy)	D_{mean} (Gy)	$V_{<95\%}$ (%)	D_{mean} (Gy)
5 mm plan							
A	63.2	70.5	0.6	50.3	56.4	1.8	57.9
B	63.1	70.5	0.7	50.2	56.5	1.7	54.2
C	62.7	70.5	0.9	48.2	56.2	3.5	43.7
D	63.0	70.4	0.7	45.7	55.8	5.2	36.4
2 mm plan							
A	64.8	71.2	0.2	51.2	56.5	1.2	56.6
B	64.4	70.1	1.4	51.2	56.4	1.2	48.8
C	63.5	71.0	1.3	49.0	56.4	3.6	37.6
D	63.6	70.1	1.4	48.8	56.3	3.5	36.3

Abbreviations: PTV_{prim} = primary PTV; PTV_{elec} = contralateral elective PTV; D_{min} = minimal dose (Gy) to 1 cc of the volume; D_{mean} = mean dose (Gy); $V_{<95\%}$ = volume (%) that received <95% of the prescribed dose.
The maximum dose to the PTVs remained constant in the various plans.

excluded and two were excluded with the use of a 2 mm margin, due to an underdosage of the primary PTV. The 40 Gy limit for the mean cSMG dose in plan D resulted in (on average) 85 to 90% of the prescribed dose being delivered to 99% of the volume of the contralateral electively treated PTV, for both margins, while the other PTVs were correctly covered (see *Table 3*).

Discussion

The mean dose to the cSMG in oropharyngeal cancer patients could be reduced from 54 Gy to approximately 40 Gy with an acceptable dose coverage of the contralateral elective PTV. Plans A and B (*Table 1*) were both consistent with the current clinical constraints for PTV dose coverage. The efforts in sparing the cSMG within the constraints of plan B led to an average dose reduction of a few Gy compared with the plans without cSMG sparing (plan A). The reduction in the contralateral elective PTV dose coverage from 95 to 90% (plan C) of the prescribed dose (54 Gy) resulted in an average mean cSMG dose of approximately 40 Gy.

In the average value of the mean cSMG dose in plan C, one plan was excluded for both margin plans. In the averages of plan D, four plans were excluded for the 5 mm margin plans and two for the 2 mm margin plans. In these plans, the dose to the primary (tumor) PTV was seriously reduced as a result of cSMG sparing, which occurred because the PLATO planning system does not use hard constraints. A correlation between the patients' TNM stage and the excluded plans could not be found. The anatomical orientation of the PTVs to the cSMG is probably of more influence. Since plan D was excluded most often, it can be concluded that it is not possible to reduce the dose to the cSMG below 40 Gy in all patients, while adequately covering the primary PTV. Furthermore, a mean cSMG dose of approximately 50 Gy in plan C is an indication of underdosage to the primary PTV in plan D. The division of the salivary glands in two parts in order to maximally spare the salivary glands was required because the planning system cannot reduce the mean dose to an OAR. A distance of 15 mm was chosen to divide the glands, at which the dose gradient can theoretically

decrease from 54 Gy to 0 Gy. This resulted in a part of the salivary glands that was adjacent to or overlapping with the target volumes and was difficult to spare. The remaining part could be spared rather easy in order to reduce the mean dose to the whole gland.

In 2008, the PTV-CTV margin applied at our department was equal to 5 mm. In this planning study, a margin of 2 mm was chosen between this margin and a theoretical margin of 0 mm. Several studies have reported margins in head-and-neck cancer patients ranging from 1.5 to 5 mm [24-26]. The use of cone-beam CT position verification has made it feasible to use a margin of 2-3 mm, which is well within this range. Reducing the PTV margin from 5 to 2 mm resulted in a few Gy extra reduction of the mean cSMG dose. This reduction is in agreement with that for the parotid gland (1.3 Gy per mm margin reduction) as described by van Asselen *et al*. [20].

The underdosage of the target volumes was restricted to the contralateral elective PTV and only occurred in the CTV-PTV margin volume adjacent to the cSMG. The reduction in dose coverage to the contralateral elective PTV from 95 to 90% of the prescribed dose (54 Gy) corresponds to reducing the minimal dose to 99% of the PTV volume by 2.7 Gy. Eisbruch *et al*. [27] denoted the level IIa lymph node area, which contains the jugulodigastric lymph nodes, as the highest risk area for subclinical lymph node metastases in oropharyngeal cancer. The dose to the contralateral level IIa region (CTV) was used as a planning constraint. It was always within the clinically accepted dose range in this study.

As of 2012, the prescribed dose to the elective nodal PTV using primary accelerated RT for oropharyngeal cancer was reduced at our department from 54 to 51 Gy in 30 fractions. When this new dose prescription is applied to the results from the planning study, the cSMG dose reductions obtained in plan C come without significant underdosage of the contralateral elective PTV in almost all patients (*Table 2*).

Of note, indications for elective treatment of the contralateral neck in oropharyngeal cancer are not similar across RT departments nation- and worldwide. Jensen *et al*. [11] showed that only ipsilateral treatment

in selected oropharyngeal cancer patients resulted in the same loco-regional control and overall survival when compared with bilateral treatment. This retrospective study was not randomized, however, and the number of patients with unilateral treatment, where bilateral treatment would have been the standard, was small. Saarilahti *et al*. [10] demonstrated that cSMG sparing resulted in better unstimulated whole salivary flow rates compared with patients receiving only parotid gland sparing IMRT. cSMG sparing was not associated with cancer recurrences within the spared volume. Both studies need to be validated in a randomized setting in larger patient cohorts. A theoretical study by De Kruijf *et al*. [12] indicated that locally underdosing the elective PTVs in head-and-neck cancer patients is associated with a decrease in subclinical disease TCP (tumor control probability) of typically a few percent.

The results from this theoretical planning study will lead to a prospective clinical study investigating cSMG sparing in oropharyngeal cancer patients. A local underdosage in the CTV-PTV margin adjacent to the cSMG (equivalent to a dose coverage of 90% in stead of 95% of the prescribed dose to the contralateral elective PTV) could be an acceptable compromise to achieve cSMG sparing. Due to this concession however, there is a chance that the recurrence rate will rise. The number and location of recurrences will therefore be monitored strictly.

In conclusion, reducing the mean dose to the cSMG from 54 Gy to values below 40 Gy is theoretically possible in patients with oropharyngeal cancer. It is associated with a slight local underdosage in the overlap area of the contralateral elective PTV and the cSMG to 90% of the prescribed dose.

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KORA

8 Sparing the contralateral submandibular gland in oropharyngeal cancer patients

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Submitted

Abstract

Purpose: To prospectively determine the prevalence of patient-reported xerostomia after sparing the contralateral submandibular gland (cSMG) in oropharyngeal cancer patients without contralateral lymph node metastases.

Patients and methods: 50 oropharyngeal cancer patients (cT1-4N0-2bM0) treated were treated with an optimized IMRT-technique with the intention to spare both parotid glands (PGs) *and* the cSMG (aim cSMG <40 Gy; cSMG-sparing cohort). They were compared with a historical cohort of 52 patients that received only PG-sparing IMRT (PG-sparing cohort). cSMG- and PG flow rates were measured 6 weeks and 1 year post-RT and converted into the percentage of baseline. Patient-reported xerostomia was recorded using the EORTC QLQ-H&N35 single items xerostomia and sticky saliva.

Results: cSMG mean dose could be reduced below 40 Gy in 50% and 21% of the patients in the cSMG-sparing and PG-sparing cohorts (mean cSMG dose 39.1 vs 50.4 Gy) respectively. cSMG flow ratio, complication rate and xerostomia scores 1 year post-RT were slightly better in the cSMG-sparing cohort (ns). At 1 year, 56% of the patients from the cSMG-sparing cohort still reported grade 2-3 xerostomia.
Post-hoc, patients were re-grouped according to mean cSMG dose above (n = 66) or below (n = 36) 40 Gy. All patients but one in the <40 Gy group had a small (T1-T2) tumor and 53% received only unilateral neck-RT. Significantly higher cSMG flow ratios at 6 weeks and 1 year post-RT in the <40 Gy group translated into lower xerostomia scores at both timepoints (at 1 yr: 67 vs. 42% grade 2-3 xerostomia, $p = 0.07$). PG function (1 yr) was similar in both groups.

Conclusions: cSMG-sparing in oropharyngeal cancer patients (N-stages ≤N2b) is challenging. In particular, it could be achieved in patients with smaller primary tumors (T1-T2) and in patients that received only unilateral neck irradiation as part of their treatment. cSMG mean doses below 40 Gy resulted in reduced patient-reported xerostomia.

Introduction

Reducing xerostomia has been an important goal of intensity-modulated radiotherapy (IMRT) for head-and-neck cancer (HNC) due to its high impact on quality of life (QoL) of patients [1,2]. Although the initial focus was on sparing parotid gland (PG) function after radiotherapy (RT), gains in patient-reported xerostomia have been absent or relatively small [3-5]. Because of the functional characteristics of the submandibular gland and the mucous content of its saliva, this gland appears more important with respect to the subjective sense of moisture [6,7]. Hence, patients could potentially benefit from its sparing with IMRT. With advancing radiation techniques for HNC, the focus in xerostomia research has therefore shifted towards additional sparing of the contralateral submandibular gland (cSMG) [8-10].

Sparing of the cSMG in head-and neck cancer is challenging however, due to its location adjacent to the level II lymph node region in the contralateral neck. Lymph nodes in this region are at risk for microscopic disease especially in cancers of the oropharynx and are usually encompassed in the contralateral elective nodal clinical target volume (CTV). In patients without contralateral lymph node metastases, advanced IMRT techniques have made it possible to spare the cSMG without significant compromise to the contralateral nodal CTV dose coverage [11,12].

In this prospective cohort study, we aimed at sparing both the PGs and the cSMG in oropharyngeal cancer patients without contralateral lymph node metastases in order to decrease the rate of patient-reported xerostomia. Dose-response relationships for the submandibular gland are published separately [13].

Patients and methods

This is a prospective (observational) cohort study. In total, 102 patients with a squamous cell oropharyngeal carcinoma (TNM stages T1-4N0-2bM0; TNM-classification, 7th edition, 2010) were consecutively included in salivary gland function studies at our department. The diagnosis was histologically confirmed in all patients. Patients with evidence

of distant metastatic disease were not included in the study and a World Health Organization status of 0 to 1 was required. No previous radiotherapy of the salivary glands was allowed nor did any patient have a malignancy or other disease of the salivary glands. Patients that underwent an ipsilateral neck dissection including the ipsilateral SMG prior to radiotherapy were included in the study.
All studies described were approved by the Medical Ethics Committee of the University Medical Center Utrecht. Informed consent was obtained from each patient.

Patients and radiotherapy
From February 2009 to July 2012, a total of 50 oropharyngeal cancer patients were treated with an optimized IMRT-technique with the intention to spare both PGs and the cSMG [11]. Patients with contralateral nodal disease or bulky neck nodes larger than 6 cm were specifically excluded from the study (N-stages N2c and N3). Indications for elective treatment of the contralateral level II-IV lymph node regions were N2a-N2b nodal stage, a tumor crossing the midline or a base of tongue carcinoma.
Gross tumor volume (GTV) delineation was performed on contrast-enhanced CT images with 3 mm slice thickness in the treatment position with an immobilization mask. The data of MRI- and PET-scans of the head-and-neck region were routinely registered to the CT data for delineation purposes. Delineation guidelines and expansion margins for the CTVs have been published elsewhere [14]. Organs at risk (spinal cord, brain, PGs and SMGs) were delineated on every CT slice. With the use of an individual head support and conebeam-CT position verification, a planning target volume (PTV) margin of 3 mm could be applied [15]. IMRT plans were obtained using the inverse-treatment planning module PLATO-ITP (Nucletron BV, Veenendaal, The Netherlands). After 30 patients had been treated, the Monaco treatment planning system (Elekta BV, Best, The Netherlands) was implemented. Seven equidistant 6 MV photon beams were applied, starting at 0°. The mean number of segments was 84 (range 42-116).

For the purpose of this study, an optimization cost function was included to reduce the cSMG mean dose below 39-40 Gy. Above this dose, no significant recovery of SMG function was observed after RT in the study by Murdoch-Kinch *et al.* [16]. In addition, at the border of the cSMG and the contralateral level II lymph node region, the 95% isodose was allowed inside the PTV of the elective nodal CTV, but not inside the CTV itself [10]. The cost function for both PGs was aimed at reducing the mean dose below 20 Gy. To ensure strict target coverage (99% of the PTV covered by 95% of the prescribed dose), the primary PTVs' cost function received more weight than the organs at risk except for the spinal cord maximum dose. The prescribed dose to the tumor GTV was 69 Gy in 2.3 Gy daily fractions and 66 Gy in 2.2 Gy daily fractions to the CTV (simultaneous boost). The elective lymph node regions were prescribed 54 Gy in 1.8 Gy daily fractions. In case of locally advanced disease (T-stages T3-T4) concomitant chemotherapy was used. In this case, the prescribed dose to the primary or nodal GTV and elective lymph node regions was 46 Gy in 2 Gy fractions, followed by a sequential boost to the primary GTV of 24 Gy in 2 Gy fractions to a total dose of 70 Gy. Patients were treated 5 times per week.

This cohort was compared with a historical cohort of 52 consecutive patients with oropharyngeal cancer that were included in prospective PG-sparing IMRT studies at our department between 2002 and 2009 (PG-sparing cohort) [14,17,18]. Details on treatment planning and delineation have been published previously. Similar to the cSMG-sparing cohort, 69 Gy in 30 fractions (simultaneous boost) or 70 Gy in 35 fractions (sequential boost; with concomitant chemotherapy) was prescribed to the primary GTV. Inverse planned, step-and-shoot IMRT was applied with the intention to spare both PGs below 20 Gy. In this group *no* active attempt was made to spare the cSMG. The SMGs were outlined for the purpose of this study and the mean doses were calculated using the 3D dose-distributions from archived treatment plans.

The pattern of any recurrent or residual disease was recorded in both cohorts.

Parotid and submandibular flow measurements

Techniques that were used for selective saliva measurements have been described previously [14,17]. Stimulated salivary flow rates were measured before treatment, at 6 weeks and 1 year after RT. Patients were instructed not to eat or drink 60 minutes before saliva collection. To avoid the influence of diurnal variation in salivary flow, consecutive measurements were scheduled as much as possible at the same daytime for each patient. The collected saliva samples were weighted and converted to ml/ min, assuming a specific gravity of 1.0 g/ ml.

Citric acid solution (5%) was applied on the mobile part of the tongue every 60 seconds, for 10 minutes. PG saliva was selectively collected using Lashley cups. Saliva near Wharton's duct orifices in the floor of the mouth was collected by gentle suction with a micropipette. This represents predominantly SMG saliva but also small secretions from the sublingual glands (SLGs), which exit nearby or through the same orifice. Because of the close proximity of both duct orifices, only the combined output of the bilateral SMGs could be measured. As a result of this, the pre-RT SMG flow rate was halved to represent the output per gland unless the patient underwent an ipsilateral neck dissection pre-RT (including the ipsilateral SMG). At 6 weeks and 1 year after RT, all measured SMG saliva was assumed to be produced by the cSMG (the mean dose to the ipsilateral SMG was on average 68 Gy and >50 Gy in all but two cases).

The cSMG flow rate at 6 weeks and 1 year after RT was converted into the percentage of baseline (unilateral) SMG flow rate. For statistical analysis, a cSMG complication was defined as cSMG flow ratio <25% of the pre-RT flow rate, or grade 4 xerostomia according to the Radiation Therapy Oncology Group/ European Organization for Research and Treatment of Cancer (RTOG/ EORTC) Late Effects Consensus Conference [19].

Assessment of patient-reported xerostomia

In order to assess patient-reported xerostomia, all patients were asked to complete the EORTC QLQ-H&N35 questionnaire at the same timepoints at which saliva was collected. This module is used for the

assessment of treatment-related symptoms in patients with HNC and contains a single symptom item on the sensation of dry mouth and a single item on sticky saliva [20]. All items are rated on a four-point Likert scale with higher scores representing worse symptoms. For the purpose of this analysis, we dichotomized xerostomia and sticky saliva into 'none or mild' (grade 0-1) and 'severe' (grade 2-3).

Statistical analysis

Baseline patient characteristics were reported using descriptive statistics (mean, median, ranges or proportions; where appropriate). Differences in cSMG and PG mean dose between groups were analyzed using Student's *t*-test. Differences in flow ratios between groups were tested using the Mann-Whitney *U* test because of nonparametric data distributions. Fisher's Exact test was used to compare proportions within cross tabulations. Patients that were lost to follow-up, missed their appointment or had recurrent illness were blinded in the analysis. All analyses were performed using IBM SPSS Statistics version 20 (IBM Corporation, Armonk, NY, USA). A *p*-value of <0.05 was considered statistically significant.

Results

Baseline characteristics across cohorts are shown in *Table 1*. The cSMG-sparing cohort contained significant more patients with node-positive disease of the ipsilateral neck, although this was not reflected in different treatment strategies with respect to uni- or bilateral (elective) neck irradiation (22% vs 15% of patients received unilateral neck RT in the cSMG-sparing and PG-sparing cohorts respectively, $p = 0.45$).

At 6 weeks after RT, 46 patients were available for analysis in both groups. At 1 year after RT, 26 patients could be analyzed in the cSMG-sparing cohort versus 37 patients in the PG-sparing cohort. Fourteen patients in the cSMG-cohort had not completed 12 months follow-up at the time of analysis.

Table 1 Patient, tumor and treatment characteristics, n (%).

	cSMG-sparing IMRT *(n=50)*	**PG-sparing IMRT** *(n=52)*	***p*-value**
Gender (male)	33 (66)	29 (56)	0.32
Age (mean; range)	60 (43-81)	58 (43-75)	0.31
T-stage			0.82
T1-T2	37 (74)	40 (77)	
T3-T4	13 (26)	12 (23)	
N-stage			0.004*
N0	7 (14)	21 (40)	
N1	17 (34)	10 (19)	
N2a-N2b	26 (52)	21 (41)	
Surgery			0.61†
Tumor ± iND	2 (4)	1 (2)	
iND only	8 (16)	7 (14)	
None	40 (80)	44 (85)	
Chemotherapy	13 (26)	7 (14)	0.14

Abbreviations: cSMG = contralateral submandibular gland; PG = parotid gland; iND = ipsilateral neck dissection (including the ipsilateral SMG).
* *p*-value for the subcohort difference N0 versus N+ (N1-N2b) using Fisher's Exact test.
† *p*-value for the subcohort difference no surgery versus surgery (tumor resection and/ or ipsilateral neck dissection) using Fisher's Exact test.

Salivary flow rates and patient-reported xerostomia

Both the cSMG and PG mean doses were significantly lower in the cSMG-sparing compared with the PG-sparing cohort (*Table 2*). Half of the patients in the cSMG-sparing cohort had their cSMG mean dose actually reduced below 40 Gy. This did not translate into significant higher cSMG flow ratios in the cSMG cohort or lower percentages of complications (cSMG flow ratio <25% of baseline) at any timepoint after RT. The cSMG-sparing cohort did show a slight increase in median cSMG flow ratio between 6 weeks and 1 year post-RT. PG function in both cohorts recovered similarly at 1 year after RT. Patient-reported xerostomia, as measured with the EORTC QLQ-H&N35 single items xerostomia and sticky saliva, was comparable in both cohorts (*Table 4*; left columns). Only at 6 weeks after RT did the cSMG-sparing cohort show a trend towards less severe complaints of sticky saliva compared with the PG-sparing cohort (51 versus 70%; $p = 0.08$).

Table 2 cSMG and PG mean dose (in Gy), flow ratios (median, interquartile range) and complication rates per treatment cohort.

	cSMG-sparing IMRT *(n=50)*	**PG-sparing IMRT** *(n=52)*	***p*-value**
cSMG mean dose (SD)	39.1 (15.7) Gy	50.4 (13.7) Gy	0.0001
cSMG <40 Gy, n (%)	25 (50)	11 (21)	0.003
cSMG flow ratio 6w (%)	6.4 (0.0-48.1)	14.2 (0.77-28.2)	0.71
cSMG flow ratio 1y (%)	11.6 (0.0-56.6)	4.9 (0.0-32.6)	0.40
cSMG complications 6w	63 %	74 %	0.37
cSMG complications 1y	65 %	76 %	0.41
PG mean dose (SD)	30.0 (12.7) Gy	34.6 (12.4) Gy	0.01
PG flow ratio 6w (%)	0.0 (0.0-59.9)	0.0 (0.0-33.3)	0.48
PG flow ratio 1y (%)	30.2 (0.0-148.5)	43.7 (0.0-117.3)	0.59
PG complications 6w	62 %	68 %	0.50
PG complications 1y	49 %	36 %	0.23

Abbreviations: cSMG = contralateral submandibular gland; PG = parotid gland; 6w = 6 weeks post-RT; 1y = 1 year post-RT.

Post-hoc analysis according to cSMG mean dose

To fully understand the effect of lower cSMG mean doses on patient-reported xerostomia, patients were re-grouped according to cSMG mean dose below or above 40 Gy, irrespective of cohort (*Table 3*). These groups did not differ with respect to sex, age, N-stage or rate of surgery. Not surprisingly, the non-spared (>40 Gy) group contained more locally advanced tumors (T3-4; 36 vs 3%, $p = 0.0001$) and consequently chemotherapy was used more often (29 vs 3%, $p = 0.001$). In addition, all patients that were eligible for only unilateral neck irradiation had their cSMG spared below 40 Gy, compared with 21% of the bilaterally treated patients ($p = 0.0001$).

In this setting, the lower mean cSMG dose in the spared group (mean 27.6 vs 54.3 Gy) did translate into significant higher cSMG flow ratios and lower complication rates 6 weeks and 1 year after RT, compared with the non-spared group (*Table 3*). cSMG function in the non-spared group clearly worsened between 6 weeks and 1 year, while PG function

Table 3 cSMG and PG mean dose, flow ratios (median, interquartile range) and complication rates for the spared (<40 Gy) and non-spared (>40 Gy) cSMG subgroups.

	cSMG dose <40 Gy *(n=36)*	**cSMG dose >40 Gy** *(n=66)*	***p*-value**
cSMG mean dose (SD)	27.6 (11.0) Gy	54.3 (8.1) Gy	0.0001
cSMG flow ratio 6w (%)	23.5 (0.0-88.2)	8.6 (0.0-22.3)	0.042
cSMG flow ratio 1y (%)	50.6 (11.4-89.8)	3.5 (0.0-10.4)	0.0001
cSMG complications 6w	53 %	78 %	0.02
cSMG complications 1y	39 %	90 %	0.0001
PG mean dose (SD)	27.6 (14.5) Gy	34.9 (10.9) Gy	0.0001
PG flow ratio 6w (%)	25.2 (0.0-85.6)	0.0 (0.0-24.4)	0.0001
PG flow ratio 1y (%)	36.0 (0.0-146.0)	40.7 (0.0-113.6)	0.93
PG complications 6w	48 %	75 %	0.001
PG complications 1y	43 %	40 %	0.84

Abbreviations: cSMG = contralateral submandibular gland; PG = parotid gland; 6w = 6 weeks post-RT; 1y = 1 year post-RT.

recovered to the same level in both groups. The significantly improved cSMG function at doses below 40 Gy influenced xerostomia *(Table 4*; right columns). Both at 6 weeks and 12 months post-RT, the spared group reported less severe complaints of dry mouth and sticky saliva. At 12 months, 42% of the patients complained of severe dry mouth versus 67% in the non-spared group, the difference approaching the statistical significance level ($p = 0.07$).

Table 4 Analysis of patient-reported xerostomia scores using the EORTC QLQ-H&N35 single items xerostomia and sticky saliva. On the right, patients were analyzed according to mean cSMG mean dose below or above 40 Gy.

EORTC gr 2-3	**cSMG-sparing** *(n = 50)*	**PG-sparing** *(n = 52)*	***p*- value**	**cSMG <40Gy** *(n = 36)*	**cSMG >40Gy** *(n = 66)*	***p*- value**
xero 6w (%)	56	60	0.68	46	66	0.08
xero 1y (%)	56	60	0.81	42	67	0.07
sticky 6w (%)	51	70	0.08	36	77	0.0001
sticky 1y (%)	30	26	0.78	13	35	0.08

Abbreviations: cSMG = contralateral submandibular gland; PG = parotid gland; 6w = 6 weeks post-RT; 1y = 1 year post-RT; EORTC gr 2-3 = Grade 2-3 complaints according to the EORTC QLQ-H&N35; xero = dry mouth; sticky = sticky saliva.

Recurrence patterns

Because of the close proximity of the cSMG to the level II lymph node region, an increased risk of recurrence may exist in the contralateral neck associated with cSMG-sparing. We evaluated the recurrence patterns in both cohorts. In the PG-sparing cohort, with a median follow-up of 70 months (range 6-111); 7 patients had a local(-regional) recurrence, involving the ipsilateral neck in one patient and the contralateral neck in another. One patient developed distant metastases. In the cSMG-sparing cohort (median follow-up 23 months; range 7-40) three patients developed a local(-regional) recurrence, involving the ipsilateral neck in one patient. One patient developed distant metastases. Two patients in this cohort had residual disease after RT, one with conversion of the contralateral neck. This latter patient did receive bilateral neck irradiation as part of RT but the cSMG could not be spared (mean dose 55 Gy). The dose coverage of primary and elective PTVs was adequate.

Discussion

In this prospective analysis, we report our initial experience at improving patient-reported xerostomia with an optimized IMRT-technique aimed at sparing both PGs and the cSMG in oropharyngeal cancer. Sparing the cSMG in this setting is challenging and could be achieved in 50% of the patients, all but one with a relatively small (T1-T2) tumor. The cSMG-sparing cohort (mean cSMG dose on average 39 Gy) showed only a marginal and non-significant improvement in the rate of patient-reported xerostomia. However, in patients where the cSMG mean dose was actually reduced below 40 Gy, a significant higher cSMG output translated into 25% less severe patient-reported xerostomia at 1 year after RT.

Several studies have reported on xerostomia after sparing the cSMG, either observer-rated [9] or patient-reported [8,10]. All studies showed a significant decrease in the rate of xerostomia associated with cSMG-sparing. Together with our findings, these results imply that efforts should be made to spare the cSMG below 40 Gy in addition to sparing the PGs, when this is clinically feasible. In this study, no recurrences were observed in the vicinity of the (spared) cSMGs in the cSMG-

sparing cohort. The technique therefore appears to be safe, although longer follow-up is required to draw definite conclusions.

Because the 30-40 Gy dose range is on the steep part of the cSMG NTCP-curve at 1 year after RT, a small mean dose reduction of a few Gy in this range will translate into relatively large reductions in NTCP [13,16]. In practice, sparing was mainly feasible in patients with more localized, smaller tumors (T1-T2) not crossing the midline. If the patient was also eligible for only unilateral (elective) nodal irradiation as part of RT, chances of sparing the cSMG below 40 Gy increased. This is probably also true for lowering the mean dose to the oral cavity (OC). The OC contains the minor salivary glands, which secrete most of the mucins in saliva. OC mean dose (as surrogate for minor gland dose) has been shown to significantly influence patient-reported xerostomia after RT [6,10,21].

Alternative approaches to preserving cSMG function after RT for more locally advanced tumors include surgical transfer of the cSMG to the submental space, which was shown to preserve cSMG flow after RT and decrease xerostomia [22,23]. Advanced radiation techniques such as intensity-modulated proton therapy (IMPT) [24] or the use of an MRI-accelerator for optimal online position verification [25] may provide new possibilities for the preservation of PG and SMG function after RT while improving target conformity.

Pitfalls of the current analysis include bias introduced by the non-randomized nature of the study. The study cohorts only differed with respect to nodal disease in the ipsilateral neck, but this did not translate into different treatment strategies for the contralateral neck (in terms of uni- versus bilateral neck RT).

In conclusion, sparing the cSMG in oropharyngeal cancer patients without contralateral nodal disease is challenging. It could be achieved mainly in patients with smaller primary tumors (T1-T2) and in patients that received only unilateral neck irradiation as part of their treatment. No increased risk of recurrence in the contralateral neck was observed. cSMG mean doses below 40 Gy resulted in reduced patient-reported xerostomia.

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9 Summary and general discussion

Xerostomia caused by radiation-induced damage to the salivary glands is the most frequently reported side-effect after radiotherapy (RT) for head-and-neck cancer (HNC). In our own experience, sixty-five percent of HNC patients had severe complaints of dry mouth 12 months after RT [1]. This, in turn, has a debilitating impact on the quality of life of patients [2,3]. With the advent of intensity-modulated radiotherapy (IMRT), it became possible to optimize both the tumor dose coverage and the sparing of healthy tissues like the salivary glands. The initial focus of head-and-neck IMRT was on sparing the parotid glands, mainly due to its anatomical location relative to most HNCs. There is abundant data showing the ability of IMRT to generate dose distributions that allow preservation of parotid gland function after treatment [4,5]. Gains in patient-reported xerostomia with parotid gland sparing IMRT, the most relevant endpoint in this respect, have been absent or relatively small however [6-8]. Based on the functional characteristics of the submandibular gland and the mucous content of its saliva, patients could potentially benefit more from submandibular gland sparing with respect to subjective complaints.

Salivary gland sparing RT takes the parotid and submandibular glands into account. This thesis addressed the dose-response relationships for the major salivary glands, the influence of quantitative and qualitative saliva parameters on subjective symptoms and (pre)clinical research on the feasibility and efficacy of salivary gland sparing RT on patient-reported xerostomia.

Parotid gland dose-response relationships

For RT treatment planning in general, sparing healthy tissues requires knowledge of dose-response relationships. In HNC, the parotid glands have been studied extensively for this purpose. The common finding in all these studies is the strong correlation of post-RT parotid gland function with mean dose, irrespective of the method of investigation. The publication of two differently shaped normal tissue complication probability (NTCP) curves for parotid gland function after RT led us to investigate the descriptive ability of the mean dose concept [9,10]. As illustrated in chapter 2, conventional RT (CRT) and IMRT lead to different

dose-volume histograms (DVH) for the parotid gland. Dose reduction with IMRT yields more non-uniform dose distributions and shallower DVHs, but there is no part of the parotid gland receiving no dose (as is often the case with CRT). Up to 6 months after RT, this translated into a significant higher risk of functional decline below 25% of baseline for parotid glands in IMRT-treated patients, after correcting for mean dose. In other words, early after RT, mean dose based models failed to fully describe the effects of radiation on the parotid glands.

Animal research has elegantly provided an explanation for the observed differences early after RT [11]. The rat parotid gland exhibits a so-called bath-and-shower (BAS) effect. This means that adding a subtolerance dose (bath) in a larger region adjacent to a high-dose irradiated subvolume (shower) of the parotid gland influences its response. In the study by Van Luijk *et al*. a shower dose of 30 Gy was administered to the caudal 50% of the parotid glands combined with a varying (0-10 Gy) bath dose to the cranial 50% of the glands. The threshold for the observed bath-effect was less than 1 Gy, as a bath dose as low as 1 Gy already caused an additional 40% loss of function at 60 days after irradiation. This enhanced early damage may be caused by a delayed re-supply of salivary stem cells from the bath-region to the high dose (shower) region of the parotid gland [11]. Additional studies are required to establish this concept. Dose distributions in the parotid glands obtained with head-and-neck IMRT bear resemblance to that of a bath-and-shower, most often in the medial (shower) to lateral (bath) direction. Our clinical data suggest that the BAS-effect may be present in human parotid glands early after IMRT.

Late after RT (at 1 year) the mean dose-based NTCP curves were comparable between CRT and IMRT, suggesting similar late radiation effects in the parotid gland independent of the related dose distributions. The renewal capacity of progenitor and stem cells, a marker of late injury, seems to be equally intact at 1 year. The TD_{50} (mean dose resulting in 50% complication probability) was equal to 38 and 40 Gy, for IMRT versus CRT respectively. Accordingly, in chapter 3, parotid gland dose-response data at 1 year obtained with different RT techniques could be combined to arrive at a single set of NTCP-

parameters for use in clinical practice. Combining multi-institutional experience yields larger patient data sets, which are required to reliably describe dose-response relationships for normal tissues. In this way, we were able to show that the previously established parotid gland mean dose threshold of 26 Gy [9], above which no recovery was to be expected over time, does not exist. A mean dose of 40 Gy corresponds to a 50% probability of parotid gland flow reduction to ≤25% of the pre-RT flow rate after one year. Obviously, when aiming at preservation of parotid gland function after RT, the dose received by these glands should be as low as possible. Trade-offs are inevitable to ensure optimal tumor dose coverage with IMRT, but parotid gland NTCP increases rather gradual with increasing mean dose.

Determinants of patient-reported xerostomia

Based on observations by us [12] and by others [6-8], recovery of parotid gland function after RT does not lead to (unequivocal) improvements in patient-reported xerostomia. In our clinical experience, HNC patients that were treated with parotid gland sparing IMRT still reported dry mouth symptoms, particularly at night. This is not surprising, because salivary flow during sleep is low and originates mainly from the submandibular glands [13]. Indeed, as described in chapter 4, the dry mouth sensation at night is a frequent problem after head-and-neck RT and is explained by submandibular gland dysfunction. It also severely affected sleeping in 20% of the patients, who often have a bottle of water on their nightstand to take sips when waking up because of dry mouth symptoms. Differentiating between dry mouth complaints during daytime and at night thus is important when investigating RT-induced xerostomia.

During daytime, the salivary glands are intermittently in a stimulated state around meals and in a resting state in between. Consequently, their volumetric contribution to whole mouth saliva changes continuously. Parotid gland dysfunction was shown to significantly influence dry mouth complaints during the day. Presumably this is most pronounced while eating (solid) food, as it will trigger difficulties in mastication and swallowing which requires sipping extra water or the pureeing of food.

Submandibular gland dysfunction also emerged as a contributing factor to xerostomia during daytime, although not consistently up to 1 year after RT. Their importance with respect to daytime complaints should not be underestimated. Even during daytime, the salivary glands are in a non-stimulated state most of time [14]. Second, before the start of RT, the baseline stimulated parotid gland flow rate in our study cohort was significantly lower compared with the submandibular gland flow rate (38% versus 62% of total output, respectively). These percentage contributions are frequently quoted vice versa for the parotid and submandibular gland, however. In older literature, reviewed recently, the contribution of the parotid glands to whole saliva during stimulation was stated to be *at least* 35%, but no upper limit was provided [15]. Besides intraindividual variation, the nature and length of application of the stimulus (a drop of 5% citric acid, every minute) and the duration of collection (10 minutes) could have influenced our findings. Third, apart from the quantitative contribution of the submandibular glands to (un)stimulated whole saliva, their secretion adds an important qualitative aspect to saliva. Together with primarily the minor salivary glands they produce mucins, which are presumed to play an important role in the subjective sense of moisture in between meals and at night. Salivary mucins are large glycoproteins that cover and protect the underlying mucosa. The high-molecular weight salvivary mucin MUC5B is present on mucosal surfaces and able to retain large amounts of water in its abundant sulfated and sialylated oligosaccharide groups. It contributes to a thin hydrophilic film that hydrates and lubricates the soft tissues of the mouth [16]. The parotid glands, on the other hand, do not produce mucins. The mucins present in submandibular gland saliva strengthen the case for its sparing with IMRT for HNC. Research in patients with Sjögren syndrome has shown that the sensation of oral dryness does not depend exclusively on the amount of water (or quantity of saliva), but rather on the quality of saliva and the presence of specific components [17]. Reduced sulfation of MUC5B leads to insufficient hydration of MUC5B in the secretion process of saliva. This in particular limits the capacity to retain water in the mucosa and could contribute significantly to xerostomia.

In chapter 5, we hypothesized that (sulfo)MUC5B levels in submandibular/ sublingual saliva from HNC patients treated with RT could better explain xerostomia compared with quantitative saliva (water) measurements. The pilot study describes two groups of patients that produced comparable amounts of submandibular and whole saliva 12 months after RT but differed with respect to the presence or absence of severe dry mouth complaints. A trend was shown towards higher (sulfo)MUC5B levels in patients with fewer complaints, although statistical significance was not reached. This was also the case when quantitative (flow rate) and qualitative (MUC5B) parameters were combined for each individual patient. The results indicate that the primary mechanism behind the sensation of oral dryness in RT patients may be similar to Sjögren patients and related to insufficient MUC5B production and functionality. More research in a larger cohort of RT patients is necessary to prove this concept. Of note, compared to healthy individuals, we measured over 8-fold lower levels of (sulfo) MUC5B in submandibular gland saliva of HNC patients at baseline. The reason for this is unclear. It may be related to the relative long storage of saliva samples at -20°C, although the sulfo-Lewis^{a} antigen is relatively robust. To what extent the catabolic state of many HNC patients at baseline can affect mucin synthesis (and sulfation) has not been studied.

Interestingly, a negative correlation was shown between the change in (sulfo)MUC5B from baseline (ΔMUC5B) and submandibular gland mean dose. Keeping in mind that the volumetric change from baseline generally becomes larger with increasing mean dose [18], it implies a different radiosensitivity of mucous and serous acini in the submandibular glands. This is line with studies in rhesus monkeys, whose submandibular gland serous cells were shown more vulnerable to radiation injury than mucous cells [19]. Data for human salivary glands is sparse but also supports a greater radiosensitivity of serous cells [20]. These findings, in turn, are compatible with the symptoms of thick and sticky saliva in patients during and shortly after the completion of RT, related to the faster decline in the watery content of saliva compared with the decline of the mucinous component.

Submandibular gland dose-response relationships

With the submandibular gland linked to subjective dry mouth symptoms after head-and-neck RT, its sparing with IMRT could potentially benefit patients. Because the ipsilateral submandibular gland inevitably receives high radiation doses due to its overlap with the tumor planning target volume (PTV) in most HNC, advanced IMRT-techniques are aimed at sparing the contralateral submandibular gland. Knowledge of submandibular gland dose-response relationships is crucial for treatment planning in this respect.

Relatively sparse data exist concerning dose-response relationships based on selective submandibular gland flow measurements. Murdoch-Kinch *et al*. first showed that submandibular gland salivary flow rates depend on mean dose with recovery over time up to a threshold of 39 Gy. At mean doses ≤39 Gy, but not above, stimulated flow rates recovered over time at 2.2% per month [18]. Our NTCP-models, described in chapter 6, did not identify such an absolute threshold dose. The TD_{50} at 1 year after RT was equal to 35 Gy, suggesting a comparable radiosensitivity (in terms of selective flow measurements) for the submandibular and parotid glands (TD_{50} 40 Gy) at 1 year after RT. Unlike the parotid gland however, the NTCP-curves for submandibular gland function at 6 weeks and 1 year after RT intersected at 40 Gy mean dose. The reason for this was that the 6 week NTCP-curve shhowed a plateau around 65-70% at mean doses higher than 40-50 Gy and did not approach 100%, as it did for the parotid gland [21]. In other words, the complication probability (flow decline to ≤25% of baseline) becomes larger between 6 weeks and 1 year after RT when the submandibular gland mean dose exceeds 40 Gy. Below 40 Gy, NTCP improves between 6 weeks and 1 year as shown by a rightward shift of the curve and reflected in the TD_{50} (23 and 35 Gy, respectively). This suggests a tendency towards late functional (volumetric) decline at mean submandibular gland doses above 40 Gy.

From a biological point-of-view, we may be looking at two processes combined in one NTCP-curve: the different recovery kinetics of the serous and mucinous cells in the submandibular gland, and their

secretions, after irradiation. As mentioned previously, human and animal data support a greater radiosensitivity of serous cells compared with mucous cells in the submandibular gland [19,20]. Thick and thread-forming mucous secretions can often be pipetted from the floor of the mouth in patients 6 weeks after RT. Particularly at higher doses, the watery (serous) content of saliva will have declined faster compared with the decline of the mucinous component. Eventually, the mucous acini may succumb to microvascular damage or to a lack of cells with renewal capacity (a marker of late injury) and atrophy may still appear. This could explain the worsened NTCP at 1 year after doses exceeding 40 Gy. There will be a great degree of variation in the extent of damage from patient to patient. This hypothesis is difficult to test directly in humans however, because it requires serial morphological studies of salivary gland tissue after RT.

Treatment planning considerations

Based on our NTCP-analysis and the data from Murdoch-Kinch *et al*; the chance of functional recovery after RT will significantly increase if the submandibular gland mean dose is reduced below 40 Gy approximately. Because the 30-40 Gy mean dose range is on the steepest part of the NTCP-curve at 1 year, a small dose reduction of a few Gy in this range will translate into relatively large reductions in submandibular gland NTCP.

Chapter 7 addresses the feasibility of advanced IMRT to reduce the contralateral submandibular gland mean dose below 40 Gy in oropharyngeal cancer patients. The contralateral submandibular gland is located directly anterior to the level II(a) lymph node region which includes the sub- or jugulodigastric lymph nodes [42]. These nodes are at the highest risk for (sub)clinical metastases in oropharyngeal cancer, particularly if the primary tumor is a base of tongue carcinoma, crosses the midline or if multiple nodal metastases are present in the ipsilateral neck. In these cases, the contralateral neck is included in the radiation fields as an elective clinical target volume (CTV). A margin is added around the CTV to account for uncertainties in delineation and patient positioning (PTV). The contralateral submandibular gland will overlap

with the resulting elective nodal PTV and reducing its dose, without compromising the dose to the elective PTV, becomes difficult. If a slight local underdosage is allowed in the CTV-PTV margin at the overlap area with the contralateral submandibular gland, our planning study showed significant reductions in the mean dose to average values around 40 Gy. It was not possible to reduce the contralateral submandibular gland mean dose below 40 Gy in all patients while adequately covering the primary tumor PTV, for example in patients where the tumor crossed the midline. With the current use of an individual head support and cone-beam computed tomography (CBCT) position verification, the PTV margin has already been reduced from 5 to 3 mm [23]. More recently, the prescribed dose to the (contralateral) elective nodal PTV using primary accelerated RT for oropharyngeal cancer was reduced from 54 to 51 Gy in 30 fractions at our department. This was based on the retrospective observation of 0% isolated neck node relapses in level II(a) of the electively treated neck. These adjustments to the treatment protocol will allow mean dose reductions to the contralateral submandibular gland below 40 Gy in more patients.

Can the local underdosage (a reduction in dose coverage to the contralateral elective PTV from 95 to 90% of the prescribed dose) be justified from an oncological point-of-view in order to spare the submandibular gland? In patients with established nodal disease in the contralateral neck (N-stage N2c) obviously this is not the case. In oropharyngeal cancer patients without contralateral nodal metastases at presentation, it probably is. It should be noted here that elective treatment of the contralateral neck for the indications mentioned previously is not the standard of care at all departments. In particular, the presence of (multiple) lymph node metastases in the ipsilateral neck is not a widely accepted indication for bilateral elective treatment. Retrospective data reported by Jensen *et al*. showed that for selected patients with tonsillar cancer without involvement of the midline structures, loco-regional control and survival were not influenced negatively by only ipsilateral treatment of the neck in case of N2a-N2b nodal stage [24].

The required dose to control microscopic disease in the cervical lymph nodes is thought to be 46-50 Gy [25]. When concurrent chemotherapy is given to the patient in case of locally advanced disease, we prescribe 46 Gy (in 23 fractions of 2 Gy) to the elective nodal PTV. Using only primary RT, 51 Gy in 30 fractions of 1.7 Gy is prescribed at present, which is equivalent to 50 Gy in 25 fractions (EQD_2) but requires a longer overall treatment time. If further dose reductions are pursued, strict monitoring of recurrent disease patterns in de (contralateral) neck is required, preferably in a randomized clinical setting.

Salivary gland sparing radiotherapy

We have prospectively investigated the impact of sparing the contralateral submandibular gland on patient-reported xerostomia in oropharyngeal cancer patients without contralateral lymph node metastases. Chapter 8 describes the initial results. At the time of writing, patient inclusion was finished but follow-up had not been completed for all patients. The technique appears to be safe: no recurrences were observed in the vicinity of the contralateral submandibular gland. The recurrence pattern was similar to that of the historical (parotid gland sparing IMRT) cohort although follow-up was considerably shorter in the submandibular gland sparing cohort (median 23 versus 70 months). In accordance to initial reports, sparing the contralateral submandibular gland mean dose below 40 Gy was feasible in approximately 50% of the patients [18,26]. In our cohort, this selected group was composed out of patients with relatively small tumors, generally not crossing the midline. Additionally, all patients that were eligible for only ipsilateral (elective) neck irradiation had their contralateral submandibular gland mean dose spared below 40 Gy. By comparison, this was the case in only 21% of the bilaterally treated patients. In conclusion, patients with locally advanced tumors of the oropharynx did not benefit from this technique with respect to dose sparing below 40 Gy. This explains to a large extent the disappointing results for the cohort as a whole, with respect to preserved submandibular gland function and patient-reported xerostomia.

In a post-hoc analysis, patients (T-stages T1-T2 in all but one patient) in whom the contralateral submandibular gland mean dose could be reduced below 40 Gy, did show significantly improved submandibular function at 6 weeks and 1 year after RT. Importantly, compared to patients with a mean dose exceeding 40 Gy, the dose reduction translated into a 25% reduction of severe patient-reported xerostomia to 42% at 1 year after RT. This difference approached the statistical significance level of 0.05. The number of patients that reported severe complaints of sticky saliva was also reduced drastically, in particular early after RT. It is important to stress that parotid gland function was equally preserved at 1 year after RT in both groups. This again provides evidence for the importance of preserving submandibular gland function with respect to patient-reported xerostomia [27,28].

Notwithstanding the favorable results in selected patients, 42% still had frequent and disabling complaints of a dry mouth. Patients with locally advanced oropharyngeal cancer did not benefit at all. Continued improvements in submandibular gland sparing are desired, although it will not be feasible to spare the bilateral glands in oropharyngeal cancer (in contrast to the parotid glands in many cases). Further optimization of IMRT in order to spare the oral cavity could reduce the dose received by the minor salivary glands [29]. In oropharyngeal cancer this will be even more challenging, due to the proximity of the primary tumor. The minor glands produce around 70% of the mucins in human saliva and have been linked to patient-reported xerostomia [28,30]. Sparing an extra organ at risk with IMRT will also come at the expense of (modestly) higher doses in other healthy organs however. For that reason, the limits of photon-IMRT with respect to sparing all salivary structures in oropharyngeal cancer may be reached in the near future.

Finally, it should be remembered that recording subjective complaints in patients involves numerous other variables. Age and gender play a role [31]. Character differences are involved. Patients can adapt to their disabilities in process of time. They learn to live with them and ascribe less significance to them over the years [32,33]. These factors should be kept in mind when interpreting patient-reported outcomes.

Future perspectives

Within the framework of the salivary gland function studies at the Radiotherapy department of University Medical Center Utrecht, more clinical follow-up data will become available on submandibular gland sparing IMRT. This will lead to the development of a prediction model for patient-reported xerostomia (both day and night) based on mean glandular doses and functional parameters. Mean dose to the oral cavity can serve as a surrogate for the minor salivary glands' dose and could also be incorporated in the model. In addition, more submandibular gland saliva samples will become available for MUC5B analysis, allowing further research into the dose-response relationships for mucin secretion and the relationship with subjective complaints after RT.

Promising new interventions aimed at the reduction of radiation-induced xerostomia include alternative approaches to prevent salivary gland radiation damage and therapies aimed at restoring function of previously damaged glands.
Over the past decade, Jha *et al*. [34] and Seikaly *et al*. [35] have developed and prospectively investigated the technique of surgically transferring the (contralateral) submandibular gland to the submental space prior to starting postoperative RT. In that way, the submandibular gland can be shielded from high dose radiation. Eighty-one percent of patients reported no or minimal xerostomia at median 14 months follow-up and 19% had moderate to severe xerostomia. Results in the longer term showed preservation of submandibular function in the surgically transferred group and 83% of the patients reporting a normal amount and consistency of saliva after RT. The procedure is safe and does not change the recurrence pattern. Submandibular gland transfer appeared reproducible in a RTOG-multicenter setting with 75% of the patients developing no acute signs of xerostomia [36]. Although these results are promising, the focus of head-and-neck cancer treatment has shifted in favor of chemoradiation with IMRT as primary treatment. In that case, the transfer procedure is generally not part of a definite surgical tumor resection and performing it singly is not very attractive.

In the developing world however, submandibular gland transfer could be used with conventional RT techniques for the prevention of xerostomia.

IMRT provides high conformality of dose distributions to target volumes and steep dose-gradients towards normal tissues, particularly in HNC. The accuracy of the daily setup of patients is essential to avoid target misses and overdosing organs at risk. Image-guided radiotherapy (IGRT) involves repeated imaging in the course of fractionated radiotherapy for setup purposes. In the current practice, cone-beam (megavoltage) CT is used for image guidance daily or weekly, depending on local standards. With the introduction of repeated scans, the assessment of volumetric changes in the patients' anatomy during RT has become available. Significant shrinkage and medial movement of the parotid glands during treatment has been shown. Consequently higher doses are received by the parotid glands than is predicted by the initial DVH from the planning CT-scan [37]. Irradiated submandibular glands have also been shown to shrink and move upward during RT [38]. This implies that the actual tolerance doses of the salivary glands may be a little higher than is expected by analyzing only planning DVH data, as was the case in our studies. Selective replanning may be performed to allow for optimal sparing of the salivary glands, but the added value of IGRT with cone-beam CT has not been unequivocally established in this respect [37,39].

Magnetic resonance imaging (MRI) is the preferred imaging modality for IGRT because of its superior soft tissue contrast. The combination of an MRI scanner with a linear accelerator for online position verification is not straightforward because of the influence of the magnetic field on the dose distribution. It does not compromise the ability to achieve IMRT dose distributions for tumors in the head-and-neck area, however [40]. A clinical prototype of the integrated MRI-accelerator system for radiotherapy is currently under development at University Medical Center Utrecht [41]. MRI-guided radiotherapy for HNC offers not only optimized position verification for the primary tumor and nodal metastases (preventing target misses) but could also improve the irradiation of the elective nodal regions. Using MRI, the

lymphatics of the head-and-neck can be visualized in more detail, which would allow the treatment of elective lymph node regions to be tailored to each individual patient. In contrast to the current standard of a CT-based atlas delineation, smaller elective volumes could be irradiation. Whether this will improve the possibilities for salivary gland sparing needs to be investigated.

Irradiation with protons has important advantages compared with the current use of photons in head-and-neck IMRT. Proton beams are typically manipulated to generate a spread-out Bragg-peak across the PTV, followed by a rapid fall-off behind the tumor to nearly zero dose. This is extremely useful in HNC, where irregularly shaped PTVs close to organs at risk have to be irradiated [42]. Intensity-modulated proton therapy (IMPT) provides excellent PTV coverage and dose homogeneity in oropharyngeal cancer, while theoretically allowing for better sparing of salivary gland tissue compared with IMRT. This was shown for the parotid glands, contralateral sublingual gland and oral cavity (minor glands) but initially not for the submandibular glands [42]. Dose and (theoretical) NTCP reductions varied widely among patients, but at least an additional 10% reduction in patient-reported xerostomia is expected with IMPT compared with IMRT. Using IMPT with a reduced spot size in oropharyngeal cancer may further enhance the benefit in terms of quality of life, because it does substantially reduce the mean dose and NTCP of the contralateral submandibular gland. In 80% of the patients, the mean dose to the contralateral submandibular gland could theoretically be reduced below 39 Gy [43]. In the latter theoretical scenario, the NTCP for moderate to severe xerostomia at 6 months after IMPT would decrease to 27% on average. These findings do require validation in clinical studies.

In those cases where significant radiation induced salivary gland damage cannot be prevented despite improvements in RT delivery, restorative therapies may salvage the patient from severe xerostomia. Acupuncture [44] and treatment with hyperbaric oxygen [45] have been reported to improve whole salivary flow rates and alleviate xerostomia after RT, but the evidence is currently insufficient to recommend their routine clinical use.

Based on major advances made in the field of stem cell research, transplantation of salivary gland stem cells has great potential for the treatment of radiation-induced xerostomia. Interestingly, increased proliferation of stem- and progenitor cells also appears to underlie the protective effect of prophylactic pilocarpine treatment on radiation-induced damage to the parotid gland [46]. The function of irreversibly damaged mouse submandibular glands was restored after intraglandular injection of an in vitro cultured *c-Kit*$^{+}$ cell population containing salivary gland stem cells [47]. Human salivary glands contain a similar putative stem cell population as rodents, expressing *c-Kit* and capable of in vitro differentiation and self-renewal [48]. This cell population was shown to reside in the excretory ducts of submandibular and parotid glands in rodents and in humans. Ongoing research is aimed at translating these findings into a clinical application for the improvement of xerostomia in irradiated HNC patients.

Currently, a proof-of-concept clinical trial is underway investigating the use of adenoviral-mediated gene transfer in radiation-induced xerostomia. This strategy is aimed at increasing the water permeability of salivary gland ductal cells that often remain present after RT. An adenoviral vector expressing the water channel protein human aquaporin-1 (AdhAQP1) is delivered to the parotid glands via ductal cannulation [49]. hAQP1 in surviving duct epithelial cells would provide a pathway for water to follow if an osmotic gradient was produced and, consequently, increased fluid secretion from the irradiated gland would result. AdhAQP1 was effective in restoring salivary flow to near normal levels in rat [50] and miniature pig [51] animal models. The observed effects were relatively short-lived however, given the immune response to first generation adenoviral vectors. New vectors have been designed that mediate transgene expression in animal salivary glands up to 6 months.

If stable expression of hAQP1 can be achieved in human parotid (and submandibular) glands after gene transfer, it remains to be proven if there is any effect on subjective complaints and quality of life in patients. The 'saliva' secreted is not of the same composition as normal saliva [49]. Only the watery component of saliva is improved following AdhAQP1 administration and mucins will still be absent. Results from clinical trials will determine if it is a viable therapeutic option.

Conclusions

The aim of this thesis was to determine the dose-response relationships for the parotid and submandibular glands and to investigate the effect of their sparing on patient-reported xerostomia after RT in the head-and-neck region and the oropharynx in particular.

The mean parotid gland dose can be used to estimate the risk of significant functional decline (NTCP) at 1 year after RT. Parotid gland NTCP at 1 year increases gradually with increasing mean dose, with no threshold dose present. Early after RT, mean dose based models do not fully describe the effects of radiation on the parotid glands.

The submandibular glands produce most saliva in the unstimulated state between meals and at night. Decline in submandibular gland function after RT is particularly linked to complaints of dry mouth at night and the associated sleep disturbances. In contrast to the parotid glands, the submandibular glands produce mucins, which retain water and help to keep the oral mucosa in a hydrated state. This qualitative aspect of saliva may explain why, after RT for HNC, parotid gland sparing alone does not seem to improve patient-reported xerostomia in a clinically significant way. The submandibular glands can show delayed functional decline at mean doses exceeding 40 Gy, but exhibit comparable radiosensitivity (in terms of volumetric output) compared with the parotid glands at 1 year after RT.

Sparing the contralateral submandibular gland with IMRT for oropharyngeal cancer is more challenging than parotid gland sparing. It is mainly feasible in selected patients with localized oropharyngeal tumors and is not associated with increased (locoregional) disease recurrence. Compared with parotid gland sparing alone, reducing the mean dose to the contralateral submandibular gland (<40 Gy) and to both parotid glands is associated with a reduction of severe patient-reported xerostomia from 67 to 42% at 1 year after RT.

Although these results are encouraging, further reductions in patient-reported xerostomia are necessary. Preserving the (mucin-secreting) function of the minor salivary glands by reducing the mean oral cavity dose could provide an extra gain, for example. Alternatively, a

more tailored definition of the elective nodal regions (and possibly the clinical target volume) for each individual patient, based on MRI, could theoretically decrease the dose to the salivary glands. A broader consensus on the indications for irradiation of the contralateral neck in oropharyngeal cancer could also help to avoid radiation damage to the major (and minor) salivary glands in patients that are at low risk of developing metastases in the contralateral neck.

The limits of IMRT with photons to spare the entire salivary system may be in sight however, particularly in patients with locally (and regionally) advanced disease of the oropharynx. Intensity-modulated therapy with protons (IMPT) can theoretically decrease the dose to the contralateral submandibular gland by on average 15 Gy in patients with oropharyngeal cancer, compared with IMRT. This would reduce submandibular gland NTCP by at least 25% on average, based on our models. Unfortunately, proton therapy is not available in The Netherlands at present. After its clinical introduction, feasibility studies should be initiated to investigate the impact of IMPT on salivary gland function and patient-reported xerostomia in patients with oropharyngeal cancer. Currently, further optimization of photon-IMRT and MRI-guided definition of target volumes are the most promising steps towards preventing radiation-induced xerostomia in patients with HNC and oropharyngeal cancer in particular. In the most advanced cases where salivary gland function cannot be spared responsibly, post-RT regeneration of salivary gland tissue with the use of stem cells may prove a viable option for the treatment of radiation-induced xerostomia.

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KORA

10 Nederlandse samenvatting

In 2010 werd in Nederland bij ongeveer 2900 patiënten een kwaadaardige tumor in het hoofd-halsgebied gediagnosticeerd. Het betreft daarmee ongeveer 3% van het aantal nieuw gediagnosticeerde maligniteiten per jaar. De belangrijkste pijlers van behandeling bij een hoofd-hals tumor zijn bestraling (radiotherapie) en chirurgie. Radiotherapie (RT) kan worden toegepast in de postoperatieve setting of als primaire behandeling en werkt door het veroorzaken van irreversibele schade aan het DNA van een tumorcel. De tumorcel zal hierdoor bij een volgende deling te gronde gaan. Om het effect van RT op de tumorcel te versterken en om mogelijke microscopische verspreiding van kankercellen (metastasen) elders in het lichaam te bestrijden, wordt soms gelijktijdig chemotherapie toegediend.
Afhankelijk van de tumorkenmerken en de aan- of afwezigheid van lymfkliermetastasen in de hals, wordt RT toegepast op zowel de primaire tumor (of het postoperatieve tumorbed) als op specifieke lymfklierstations in de hals. De doelgebieden bij RT in het hoofd-halsgebied liggen door de complexe anatomie vrijwel altijd nabij gezonde organen zoals de speekselklieren, slikspieren, ruggenmerg, stembanden, huid en de slijmvliezen van de mondholte en keel. Met name bij tumoren van het gedeelte van de keelholte dat direct achter de mondholte is gelegen (oropharynx) lopen al deze gezonde structuren kans op significante bestralingsschade.

Speeksel is essentieel voor de mondgezondheid en een vermindering in kwaliteit of kwantiteit wordt meestal direct opgemerkt. De productie van speeksel vindt voornamelijk plaats in de grote speekselklieren, te weten de oorspeekselklieren (glandula parotis) gelegen voor de oorschelp en de onderkaakspeekselklieren (glandula submandibularis) gelegen langs de onderkaak aan beide zijden. Een klein deel van het speeksel, dat zeer eiwitrijk is, wordt geproduceerd door kleine speekselkliertjes die verspreid voorkomen in het gehemelte, de binnenzijde van de lippen en de slijmvliezen in de mondholte. De speekselproductie van de parotiden kan selectief gemeten worden met speciaal ontworpen vacuümcups die over de uitgang van Stensen's duct in het wangslijmvlies kunnen worden geplaatst. De bilaterale submandibulaire speekselproductie kan met een micropipet worden

opgevangen in de mondbodem bij de uitmondingen van Wharton's duct.
Bestralingsschade aan de speekselklieren uit zich meestal al tijdens de eerste weken van een RT behandeling door vermindering van de speekselproductie. Bij hoge bestralingsdoses kunnen de speekselklieren uiteindelijk irreversibel beschadigd raken. Behalve een gevoel van droge mond, kan een verminderde speekselproductie ook leiden tot pijnlijke slijmvliezen, kauw- en slikproblemen, zweertjes in de mond en tandbederf. Secundair hieraan kunnen verminderde voedselinname en gewichtsverlies het gevolg zijn. Het hele complex van symptomen veroorzaakt door een verminderde speekselproductie wordt ook wel xerostomie genoemd. Xerostomie is de meest frequent voorkomende bijwerking na RT in het hoofd-halsgebied en heeft een significante invloed op de kwaliteit van leven van patiënten die bestraald zijn voor een hoofd-hals tumor. Omdat een effectieve therapie voor RT-geïnduceerde xerostomie (nog) niet voorhanden is, bestaat de aanpak vooral uit het voorkomen van bestralingsschade aan de speekselklieren.
De radiotherapie heeft in de afgelopen 2 decennia een belangrijke ontwikkeling doorgemaakt met de introductie van intensiteit-gemoduleerde radiotherapie (IMRT). Deze computer-geoptimaliseerde 3D-techniek maakt het mogelijk om de tumor tot een hoge(re) dosis te bestralen, terwijl omliggende gezonde weefsels zoals de speekselklieren beter kunnen worden gespaard. Speekselklier sparende radiotherapie is erop gericht om de tumor optimaal te behandelen en ernstige xerostomie klachten zoveel mogelijk terug te dringen. Om hoofd-hals IMRT hierbij effectief toe te kunnen passen is het van belang om de tolerantiedoses van de (grote) speekselklieren te kennen en te weten welke speekselklier parameters bijdragen aan het ontstaan van xerostomie na RT.

Het onderzoek naar de preventie van xerostomie met hoofd-hals IMRT heeft zich initieel vooral gericht op het beperken van de bestralingsdoses in de glandula parotis. Door zijn anatomische ligging konden deze speekselklieren relatief eenvoudig gespaard worden met IMRT, zonder

onderdosering van de primaire tumor en de electieve lymfklierstations. Voor het sparen van gezonde weefels met IMRT is het belangrijk om de dosis-respons relatie te kennen. Normal tissue complication probability (NTCP) modellen worden gebruikt om de sigmoïdale dosis-respons relatie te beschrijven tussen de (gemiddelde) bestralingsdosis in een gezond orgaan enerzijds en de kans op significant functieverlies anderzijds. **Hoofdstuk 2** beschrijft de NTCP- curves voor de glandula parotis na bestraling met conventionele (2D) RT technieken versus IMRT. Bij analyse van een grote groep patiënten blijkt dat, gecorrigeerd voor de gemiddelde bestralingsdosis, in de eerste 6 maanden na RT een grotere kans op significant functieverlies van de parotis (<25% ten opzichte van baseline) aanwezig is na IMRT. Hierbij moet worden opgemerkt dat de gemiddelde dosis van individuele patiënten met IMRT wel significant lager is vergeleken met conventionele technieken. De gemiddelde dosis in de parotis is dus niet de optimale parameter om de bestralingsreactie in de glandula parotis te voorspellen tijdens de eerste maanden na RT. De spatiële dosisverdeling in de glandula parotis lijkt hierbij dus een rol te spelen. Eén jaar na RT blijken de NTCP-curves gebaseerd op de gemiddelde dosis wel vergelijkbaar tussen beide technieken. De TD_{50} (de gemiddelde dosis die gelijk staat aan 50% kans op significant functieverlies in de parotis) was 38 en 40 Gray (Gy) respectievelijk met IMRT en conventionele RT.
Op basis van deze bevindingen wordt in **Hoofdstuk 3** een NTCP-curve beschreven, waarmee met de gemiddelde dosis de kans op significant functieverlies van de glandula parotis 1 jaar na RT kan worden afgelezen. Hiervoor werden dosis-respons gegevens gebruikt van ruim 200 patiënten die behandeld werden op de afdelingen Radiotherapie van het UMC Utrecht en de University of Michigan in Ann Arbor, Verenigde Staten. De analyse toont aan dat de door Eisbruch *et al.* beschreven absolute tolerantie dosis van 26 Gy, waarboven geen herstel van de parotisfunctie te verwachten zou zijn, niet bestaat. De curve laat een geleidelijke toename zien van de kans op significant functieverlies bij een hogere gemiddelde parotisdosis, zonder drempelwaarde. Een gemiddelde dosis van 40 Gy in de parotis correspondeert met een 50% kans op functieverlies van de parotis tot <25% van baseline (TD_{50}). Bij

het sparen van de parotisfunctie met hoofd-hals IMRT is een zo laag mogelijke dosis natuurlijk optimaal, maar dit kan niet ten koste gaan van de dosis in de primaire tumor en de (electieve) lymfklierstations. Uit deze studie blijkt dat er bij klinisch relevante doses tussen de 25-30 Gy slechts een 17-26% kans bestaat op significant functieverlies 1 jaar na RT.

Uit meerdere publicaties blijkt dat het sparen van alleen de glandula parotis met hoofd-hals IMRT leidt niet tot een klinisch significante vermindering van het aantal patiënten met ernstige klachten van een droge mond. Deze patiënten blijken in belangrijke mate nog klachten te hebben van een droge mond 's nachts. Dit is te verklaren uit het feit dat de speekselproductie 's nachts laag is en alleen afkomstig uit de submandibulaire speekselklieren. Ook overdag is een aanzienlijk deel van de speekselproductie afkomstig uit de submandibulaire klieren, met name tussen de maaltijden in (de niet-gestimuleerde fase). Tijdens de maaltijden zijn vooral de parotiden het meest actief, wat bijvoorbeeld merkbaar is aan het 'watertanden'.
Een nachtelijke droge mond blijkt een frequent probleem na parotis-sparende IMRT (gerapporteerd door 56% van de patiënten na 1 jaar) en wordt verklaard door een verminderde functie van de submandibulaire speekselklieren. Ongeveer 20% van de patiënten blijkt hierdoor te kampen met ernstige slaapproblemen. Dit betekent dat zij frequent wakker worden door een droge mond en altijd een flesje water op het nachtkastje hebben staan. Uit het bovenstaande, beschreven in **Hoofdstuk 4**, volgt dat het bij onderzoek naar RT-geïnduceerde xerostomie essentieel is om te differentiëren tussen droge mond klachten overdag en 's nachts.
Naast het feit dat de submandibulaire speekselklieren qua volume het meeste speeksel produceren tijdens de niet-gestimuleerde perioden 's nachts en buiten de maaltijden om, bevat submandibulair speeksel belangrijke eiwitten die bijdragen aan de bevochtiging van het mond- en keelslijmvlies: mucines. Speekselmucines, met name MUC5B, zijn grote glycoproteïnen die in staat zijn om watermoleculen te binden en daardoor bijdragen aan een dun laagje op de slijmvliezen dat zorgt

voor hydratie en lubricatie. Het gevoel van een vochtige of droge mond lijkt hier in belangrijke mate mee samen te hangen. Speeksel uit de parotiden bevat geen mucines en bestaat voor het overgrote deel alleen uit water. Dit vormt tevens een verklaring voor de bevinding dat het sparen van alleen de parotiden met hoofd-hals IMRT ontoereikend is voor het verminderen van xerostomie. De hypothese die onderzocht werd in **Hoofdstuk 5**, is dat het MUC5B gehalte in submandibulair speeksel sterker samenhangt met droge mond klachten dan het gemeten speekselvolume. In een pilot studie werden twee groepen patiënten geanalyseerd die 1 jaar na hoofd-hals RT een vergelijkbare submandibulaire en totale (plus parotis) speekselproductie hadden, maar verschilden door de aan- of afwezigheid van ernstige droge mond klachten. Patiënten zonder droge mond klachten hadden een hogere concentratie MUC5B in hun speeksel, hoewel het niet een statistisch significant verschil betrof. Ook bij het combineren van de kwantitatieve (speekselproductie) en kwalitatieve (MUC5B) parameters van elke individuele patiënt werd dezelfde trend waargenomen. Deze resultaten behoeven nader onderzoek maar wijzen op een rol van verlaagde MUC5B concentraties bij het ontstaan van droge mond klachten na RT in het hoofd-halsgebied.

Nu het belang van de submandibulaire speekselklierfunctie ten aanzien van xerostomie en droge mond klachten na RT is onderstreept, ligt het voor de hand dat patiënten met een hoofd-hals tumor baat kunnen hebben bij het sparen van deze speekselklieren met IMRT. Over de dosis-respons relatie van de glandula submandibularis na RT in het hoofd-halsgebied is in de literatuur relatief weinig gepubliceerd. Informatie uit NTCP-modellen van de submandibularis is van belang voor het sparen van deze klier met hoofd-hals IMRT. Murdoch-Kinch *et al.* toonden aan dat herstel van de submandibularisfunctie na RT afhangt van de gemiddelde dosis, echter boven een dosis van 39 Gy vonden zij geen enkel herstel van functie in de tijd. De NTCP-modellen, beschreven in **Hoofdstuk 6**, laten zien dat de TD_{50} van de glandula submandibularis na 1 jaar gelijk is aan 35 Gy en daarmee het model van de parotis benadert (TD_{50} 40 Gy). In tegenstelling tot de parotis

snijden de NTCP-curves van de submandibularis van 6 weken en 1 jaar na RT elkaar bij een gemiddelde dosis van 40 Gy. Dit komt doordat de NTCP-curve van 6 weken na RT bij doses hoger dan 40 Gy een plateau laat zien van 65-70% NTCP en niet doorloopt naar 100%, zoals dat 1 jaar na RT wel het geval is. Met andere woorden, de kans op significant functieverlies (<25% ten opzichte van baseline) wordt groter tussen 6 weken en 1 jaar na RT wanneer de gemiddelde bestralingsdosis in de submandibularis boven de 40 Gy uitkomt. De kans op significant functieverlies na 1 jaar loopt, net als voor de glandula parotis, echter maar geleidelijk op naar 100%. Onder de 40 Gy is er dus sprake van neiging tot functieherstel tussen 6 weken en 1 jaar na RT, hetgeen overeenkomt met de eerder gepubliceerde data.

Deze bevindingen zijn deels te verklaren vanuit radiobiologisch oogpunt. De glandula submandibularis bevat zowel sereuze (water-producerende) als mucineuze (eiwit- of mucine-producerende) acinaire cellen. Onderzoek bij dieren en mensen heeft een grotere (vroege) bestralingsgevoeligheid aangetoond van de sereuze acinaire cellen. Het watergehalte van speeksel neemt bij hogere bestralingsdoses daarom relatief sneller af in vergelijking met de mucineuze component. Dit is de reden dat patiënten vooral in de eerste weken en maanden na RT dan ook klagen over kleverig speeksel in de mond en keel. Op langere termijn kunnen bij hogere doses ook de mucineuze cellen hun functie verliezen of vindt er niet voldoende regeneratie plaats vanuit de stamcelpopulatie. Dit zou het functieverlies (in termen van volumetrische output) tussen 6 weken en 1 jaar bij gemiddelde doses boven de 40 Gy kunnen verklaren.

Oncologisch gezien is het sparen van de glandula submandibularis met hoofd-hals IMRT uitdagend vanwege het feit dat deze gelegen zijn naast de level II lymfklierstations aan beide zijden van de hals. De halslymfklieren worden voor de oncologische verslaglegging anatomisch onderverdeeld in meerdere levels, die ieder zorgen voor de lymfdrainage van een specifiek gedeelte van de mond- en keelholte. De level II lymfklieren draineren voornamelijk de oropharynx en tumoren in dit gebied metastaseren meestal dus als eerste naar deze lymfklieren. Ook wanneer de level II lymfklieren radiologisch niet afwijkend zijn,

worden ze vaak 'at risk' beschouwd en frequent electief meebestraald bij hoofd-hals IMRT. Bovendien ligt de glandula submandibularis aan de zijde van een oropharynxtumor (ipsilateraal) vaak zo dichtbij de tumor dat deze onvermijdelijk een hoge gemiddelde dosis ontvangt (>60 Gy). Echter, de dosis in de contralaterale submandibularis kan theoretisch worden beperkt bij patiënten waarbij geen aanwijzingen bestaan voor metastasen in de contralaterale hals. **Hoofdstuk 7** beschrijft de ontwikkeling van een IMRT-techniek voor het sparen van de contralaterale submandibularis en de beide parotiden bij patiënten met een oropharynxtumor. Gebaseerd op de hierboven beschreven NTCP-analyse en de data van Murdoch-Kinch *et al.* kan gesteld worden dat de kans op functiebehoud van de contralaterale submandibularis het grootst is wanneer de gemiddelde dosis <40 Gy is.

Bij het plannen van hoofd-hals IMRT worden de verschillende doelgebieden ingetekend op een CT- (en MRI-) scan en hieromheen wordt een marge genomen die rekening houdt met onzekerheden in de intekening en positie-onnauwkeurigheid tijdens RT (planning target volume ofwel PTV-marge). Door de ligging van de (contralaterale) glandula submandibularis in de hals, zal deze deels overlappen met het PTV van de electieve level II lymfklieren. Wanneer er in dit overlap-gebied een kleine onderdosering wordt toegestaan, bleek bij de helft van de patiënten in deze planningsstudie een gemiddelde dosis onder 40 Gy haalbaar in de contralaterale submandibularis. De dosiscoverage van de contralaterale level II lymfklieren wordt daarmee verlaagd van 95 naar 90% van de voorgeschreven dosis. Bij patiënten met grote tumoren van de oropharynx die bovendien de midline overschrijden, blijkt een dergelijke dosisreductie meestal niet mogelijk omdat de coverage van de primaire tumor niet adequaat is.

De PTV-marge die wordt toegepast bij hoofd-hals IMRT op de afdeling Radiotherapie van het UMC Utrecht is enkele jaren geleden gereduceerd van 5 naar 3 mm met het gebruik van een individuele hoofdsteun en positie-verificatie doormiddel van cone-beam CT (CBCT). Bovendien is recentelijk de (geaccelereerde) bestralingsdosis in de electieve lymfklierstations gereduceerd van 54 naar 51 Gy in 30 fracties, op basis van de retrospectieve observatie van 0% geïsoleerde lymfklierrecidieven

in de electief bestraalde (level II) hals. Door deze aanpassingen van het behandelprotocol zal de gemiddelde dosis in de contralaterale submandibularis bij nog meer patiënten onder 40 Gy kunnen worden gereduceerd.

Hoofdstuk 8 beschrijft tenslotte de eerste resultaten van een prospectieve studie met deze IMRT-techniek naar het effect van het sparen van de contralaterale glandula submandibularis op droge mond klachten bij patiënten met een oropharynxtumor. Alleen patiënten zonder radiologische aanwijzingen voor lymfkliermetastasen in de contralaterale hals werden in deze studie geïncludeerd. Bij 50 geïncludeerde patiënten werd met een mediane follow-up van 23 maanden geen geïsoleerd recidief gezien in de contralaterale (level II) hals. De IMRT-techniek lijkt derhalve veilig, echter de follow-up is op het moment van schrijven nog niet geheel afgerond. Net als in de planningstudie bleek het bij slechts 50% van de patiënten mogelijk om de gemiddelde dosis in de contralaterale submandibularis te reduceren onder 40 Gy. Dit betrof voornamelijk patiënten met kleine oropharynxtumoren die niet over de midline groeiden, en alle patiënten waarbij alleen de ipsilaterale hals (electief) werd bestraald. In vergelijking met een ouder cohort patiënten die binnen een prospectief onderzoek alleen parotis-sparende IMRT hadden gekregen, vielen de resultaten voor wat betreft functiebehoud van de glandula submandibularis en droge mond klachten dan ook tegen. Na 1 jaar had 56% van de patiënten ernstige klachten van droge mond en had 30% klachten van kleverig speeksel, hetgeen niet significant verschilt van het patiëntencohort waarbij alleen de parotiden werden gespaard (waarin bij 21% van de patiënten de gemiddelde contralaterale submandibularisdosis onder de 40 Gy bleef, zonder dat dit een vooraf gespecificeerd doel was).

Bij een post-hoc analyse van de beide cohorten gezamenlijk, waarbij de patiënten werden herverdeeld op basis van gemiddelde contralaterale submandibularisdosis boven of onder 40 Gy, werd wel een effect gezien van de dosisreductie in de contralaterale submandibularis op zowel functiebehoud als op het klachtenpatroon. De patiënten met een contralaterale submandibularisdosis onder 40 Gy hadden een

significant hoger submandibulair speekselvolume na 6 weken en 1 jaar na hoofd-hals IMRT. Dit vertaalde zich ook in een 25% reductie van het aantal patiënten met ernstige droge mond klachten na 1 jaar: 42% versus 67% bij een dosis >40 Gy. Dit verschil benadert het significantie-niveau van 0.05. Het is hierbij belangrijk om te vermelden dat de parotisfunctie in beide groepen na 1 jaar in gelijke mate hersteld was. Het aantal patiënten met ernstige klachten van kleverig speeksel in de eerste weken na RT daalde in deze analyse significant van 77% naar 36%. Patiënten met een lokaal-gevorderde oropharynxtumor (stadium T3-T4) behoorden op één na allemaal tot de groep waarbij de contralaterale submandibularisdosis boven 40 Gy uitkwam.

Conclusie en toekomst

Dit proefschrift beschrijft de dosis-respons relaties van de oorspeekelklier (glandula parotis) en de onderkaakspeekselklier (glandula submandibularis) na bestraling in het hoofd-halsgebied. Tevens werd onderzocht of het sparen van deze speekselklieren met intensiteitgemoduleerde radiotherapie (IMRT) bij tumoren van de oropharynx effect heeft op droge mondklachten (xerostomie), de meest frequent gerapporteerde bijwerking van deze behandeling.
De gemiddelde bestralingsdosis in de parotis kan gebruikt worden om het risico op significant functieverlies na 1 jaar in te schatten. Dit risico loopt geleidelijk op bij een hogere gemiddelde dosis, zonder dat er sprake is van een drempel. Voor het beschrijven van vroege schade aan de parotis is de gemiddelde dosis minder geschikt. Schade aan de parotiden uit zich overdag, aangezien deze klieren vooral actief zijn tijdens de maaltijden. Functieverlies van de glandula submandibularis na RT leidt tot droge mond klachten ’s nachts en daarbij ontstaan ook slaapproblemen. De submandibulaire klieren produceren belangrijke eiwitten (mucines) die een rol spelen bij de bevochtiging van de slijmvliezen in de mond en keel. Het sparen van alleen de parotiden met IMRT heeft mede daarom weinig effect gehad op droge mond klachten na radiotherapie in het hoofd-halsgebied.
De late bestralingsreactie in de submandibulaire klieren (1 jaar na RT) verloopt qua volumetrische output bij benadering hetzelfde als in de

parotiden. Het sparen van de submandibulaire speekselklierfunctie met IMRT bij tumoren van de oropharynx is echter veel complexer dan parotis-sparing. De ipsilaterale klier kan meestal niet gespaard worden door de nabijheid van de primaire tumor. Het sparen van de contralaterale klier blijkt met name haalbaar bij patiënten met kleinere tumoren en patiënten waarbij de hals alleen eenzijdig bestraald wordt. De reductie van de gemiddelde dosis in de contralaterale klier (tot onder 40 Gy) met IMRT, in aanvulling op het sparen van de parotiden, leidt tot een vermindering van het aantal patiënten met ernstige klachten van droge mond na 1 jaar: 67% versus 42% respectievelijk, zonder en mét sparing van de contralaterale submandibularis.

Hoewel deze resultaten bemoedigend zijn, moet het aantal patiënten met ernstige klachten van een droge mond na RT in het hoofd-halsgebied verder worden teruggedrongen. Hierbij kan gedacht worden aan verdere optimalisatie van IMRT, waarbij ook de kleine speekselklieren in de mondholte (die veel mucines produceren) zoveel mogelijk gespaard worden. Het individualiseren van de bestraling van electieve lymfklierstations, bijvoorbeeld met behulp van MRI, behoort ook tot de mogelijkheden. Voor patiënten met lokaal-gevorderde tumoren van de oropharynx zou RT met protonen door zijn bundeleigenschappen meerwaarde kunnen hebben bij het sparen van de submandibulaire speekselklier. Helaas is deze modaliteit op dit moment nog niet beschikbaar in Nederland.
Bij patiënten met een hoofd-hals tumor waarbij het sparen van speekselklieren niet mogelijk blijkt ondanks geavanceerde bestralings-technieken, vormt de regeneratie van speekselklierweefsel met stamcellen een veelbelovende therapie. Er zijn studies gaande die erop gericht zijn deze methode vanuit dierexperimenteel onderzoek te vertalen in een klinische toepassing bij bestraalde patiënten.


KORA

10 List of publications

Submitted papers

Dijkema T, Raaijmakers CP, Braam PM, Roesink JM, Terhaard CH. Sparing the contralateral submandibular gland in oropharyngeal cancer patients. 2013

Dijkema T, Terhaard CH, Braam PM, Roesink JM, Raaijmakers CP. Dose-response relationships for the submandibular gland after radiotherapy for head-and-neck cancer. 2012

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2nd ESTRO Forum 2013, Geneva, Switzerland

Dijkema T, Raaijmakers CP, Braam PM, Roesink JM, Terhaard CH. Sparing the contralateral submandibular gland in oropharyngeal cancer.
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ECCO 2007, 14th bi-annual meeting, Barcelona, Spain


KORA

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KORA

10 Curriculum vitae

Tim Dijkema was born on the 2nd of August 1980 in Leiderdorp, The Netherlands. After graduating from secondary school (atheneum) at Maasland College in Oss, he went on to study medicine at the Catholic University Nijmegen (today Radboud University Nijmegen) in September 1998. After his doctoral phase (cum laude), in May and June 2002, he travelled to Nepal for a foreign students exchange program at the Pediatric Surgery department of Kanti Children's Hospital in Kathmandu. Before obtaining his medical degree in August 2004, he wrote a graduate thesis on treatment-related toxicity in children and adolescents with acute lymphatic leukemia. The work, performed at the Pediatric Oncology department of UMC St Radboud (supervisor Prof. dr. P.M. Hoogerbrugge) was awarded the Student KOC-Award in October 2004.

As MD, he worked in pediatrics (Jeroen Bosch Ziekenhuis, Den Bosch) and as clinical research physician (Clinical Research Center Nijmegen, UMC St Radboud). His interest in radiotherapy was sparked in 2006, during a year as resident in internal medicine and medical oncology at Amphia Ziekenhuis (loc. Langendijk) in Breda.

In December 2006, the research described in this PhD thesis was initiated at the Radiotherapy department of University Medical Center Utrecht, granted by the Dutch Cancer Society (UU 2006-3573) and under supervision of dr. C.P.J. Raaijmakers, dr. C.H.J. Terhaard and dr. J.M. Roesink. In March 2008, he visited Prof. dr. A. Eisbruch at the department of Radiation Oncology at the University of Michigan in Ann Arbor, USA. The collaboration resulted in a joint publication (Chapter 3). His radiation oncology training had commenced in November 2007 and was spent at RISO Deventer (drs. E.J.A. Vonk) and UMC Utrecht (dr. C.H.J. Terhaard). From January to July 2012, he was hosted by Prof. dr. M. Verheij at the Netherlands Cancer Institute in Amsterdam for a research elective at the department of Biological Stress Response on phosphocholine-enhanced uptake of cytostatic and radiosensitizing compounds in tumor cells. His residency continues until May 2013. In July 2013, he will start working as radiation oncologist at the Radiotherapy department of Radboud University Nijmegen Medical Center.