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van den Broek, G.B.

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Promotor	prof. dr A.J.M. Balm
Co-promotores	dr C.R.N. Rasch dr M.W.M. van den Brekel
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Faculteit der Geneeskunde

Voor Dorien

Contents

CHAPTER 1

Chemoradiation for advanced head and neck cancer; an introduction

Epidemiology

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common cancer in men and accounted worldwide for approximately 540,000 new cases among men and 220,000 among females per year[1]. HNSCC is a global problem with an incidence ranging from 4-60/100,000 in males and 1-16/100,000 in females. In the European Union, approximately 74,000 new cases and about 29,000 deaths were reported in 1997, whereas in the United States over 40,000 cases are diagnosed annually[2]. In the Netherlands, the annual incidence of head and neck cancer is increasing over the years with about 2500 new patients annually, comprising 4% of all newly diagnosed cancers. The majority of head and neck malignancies are squamous cell carcinomas arising in the mucosal epithelium of the upper aerodigestive tract. The oral cavity is affected most, followed by larynx, oropharynx and hypopharynx[3]. Carcinomas of the nasopharynx, paranasal sinus, nasal cavity and middle ear are rare.

Many head and neck cancer patients still present with advanced stage III and IV disease[4]. A relative increase in patients presenting initially with stage III/IV disease has even been observed in the Netherlands, which may probably be attributed to the increasing consumption of cigarettes and alcohol over the last decades[5]. Brouha et al.[4] demonstrated a significant increase in the proportion of T4 HNSCC compared with non-T4 tumors over the period 1980 to 2000 with an estimated an increase of 0.9% per year. The increase in advanced stage HNSCC may, however, be biased by improved imaging techniques with early detection of tumor invasion in the surrounding tissue leading to upstaging of the disease. Patients with advanced disease have a worse prognosis leading to high treatment costs and poor outcome.

Resectable and irresectable head and neck squamous cell carcinoma

Patients with limited disease can be treated curatively either by surgery or radiotherapy alone with good results. On average, overall survival figures for T1 and T2 carcinomas reach 90% and 80% respectively[6]. For patients with locally advanced carcinomas surgery, radiotherapy, chemotherapy or a combination of these modalities can be used as treatment option. Anatomically unresectable tumors used to be treated by radiotherapy or chemotherapy, whereas advanced resectable tumors used to be treated by surgery and postoperative radiotherapy. However, a large group of these resectable tumors were functionally unresectable and most of these tumors had an early recurrence or poor organ function. Functional unresectability is difficult to define anatomically, but in a recent survey among Dutch Head and Neck Surgeons and Radiation Oncologists a large majority of them judged that total glossectomy and anterior mandibulectomy even with adequate reconstruction met the criteria of severe functional impairment (A. Kreeft, personal communication). When vital functions cannot be secured, the patient can be described as *functionally inoperable* and the tumor as *functionally unresectable*[7]. Criteria of anatomical unresectability are easier to define. When tumor-free resection margins cannot be obtained without inflicting, unacceptable or life threatening damage to the patient, the lesion is considered unresectable. Tumors invading essential structures like the internal carotid artery or the spinal canal are examples of anatomically unresectable lesions.

The involvement of vital functions like speech and swallowing require difficult clinical decision making in patients with carcinomas of the head and neck. Although with the use of advanced free flap reconstruction techniques, large tumors can be removed completely, the functional result cannot always be predicted and can be poor. Major resections often lead to impairment of speech, articulation, swallowing, mastication, taste and smell or a combination of these. After commando procedure for oropharyngeal carcinoma patients often experience serious problems with eating (in public) and speech intelligibility as well as facial disfigurement[8,9]. Ackerstaff et al.[8] showed that only a minority of patients returned to their jobs after major commando resections. Although in most surgically treated patients appropriate defect reconstruction may lead to an improvement of pretreatment functional levels, these pretreatment levels remain deviant from normal scores as found in the general population[10].

Induction chemotherapy and radiotherapy

Due to the impact on quality of life, treatment of head and neck cancer is multifaceted. Until the late eighties surgery, radiotherapy or a combination of both were the standards of treatment for resectable or unresectable head and neck cancer. Chemotherapy was usually advocated in patients with recurrent or disseminated disease. The last decades, evidence emerged showing that cisplatin-based chemotherapy in combination with radiotherapy could improve complete responses of primary malignancies[11-14].

Several induction chemotherapy trials were set up to determine the value of chemotherapy in treatment of head and neck cancer. The first landmark study is the Veterans Affair (VA) randomized trial comparing induction chemotherapy and radiotherapy compared to surgery with adjuvant radiotherapy[15]. Three-hundred and thirty-two patients were randomly assigned to receive either three cycles of chemotherapy and radiation therapy or surgery and radiation therapy. Clinical response was assessed after two cycles of chemotherapy. Patients with response continued to have one cycle of chemotherapy and definite radiotherapy whereas patients without response underwent surgery and postoperative radiotherapy. Preservation of the larynx was achieved in 64% of patients demonstrating that chemotherapy was capable to attain organ preservation with similar two years survival rates (68%) for both treatment arms. A comparable study including hypopharyngeal cancer was undertaken by the European Organization for Research and Treatment of Cancer (EORTC) and demonstrated a similar survival between induction chemotherapy and the surgical arms[16]. Organ preservation was achieved in one-third of patients. Other trials followed, also including oropharyngeal and oral cancer showing similar results[17,18].

In a meta-analysis of randomized chemoradiation trials, comparing induction, neo-adjuvant and concurrent chemoradiation regimens with conventional radiotherapy, a survival advantage of 8% was established in favor of concomitant chemotherapy, whereas induction chemotherapy and neo-adjuvant chemotherapy did not render a survival benefit[19]. Several explanations have been proposed for the limited success of induction chemotherapy. Firstly, successful modalities like surgery and radiotherapy are often postponed by induction chemotherapy. Secondly, most HNSCC keep their ability to regenerate and repopulate.

Thirdly, because overall treatment time is extended repopulation of surviving tumor cells may limit any advantage of the cytotoxicity of chemotherapy. Another explanation may be that chemotherapy targets tumor cells that are already sensitive to radiotherapy, and chemotherapy may not be able to overcome factors that contribute to treatment failure following radiotherapy like hypoxia. It is also possible that the induction chemotherapy induces toxicity that influences outcome negatively. Recently however, some very promising results were obtained using a combination of cisplatin, 5-FU and taxol[20,21]. Ghi et al.[20] investigated differences in outcome in 24 patients treated with docetaxel, cisplatin and 5-fluorouracil (TPF) followed by concurrent chemoradiotherapy (CHT-RT) or CHT-RT alone. The complete response rate of the neo-adjuvant treatment arm and the CHT-RT alone arm was 80% and 63%, respectively. Although no definitive answers can be given as yet, scheduling of radiotherapy and chemotherapy appears to be critical.

Concurrent chemoradiotherapy

Due to these negative results of induction chemotherapy trials, physicians continued to look for better results and included concurrent chemoradiotherapy rather than induction chemotherapy schedules into clinical trials. Several investigators hypothesized that chemotherapy and radiotherapy have a synergistic effect. This chemotherapeutic radiosensitization might be due to the ability of chemotherapy to inhibit repair of sublethal radiation damage[22,23]. Furthermore, the overall treatment time decreased in comparison with induction chemotherapy, resulting in a reduced ability of head and neck squamous cells to repopulate. The superiority of concurrent chemoradiotherapy (CCRT) was demonstrated by the Radiation Therapy Oncology Group and the Head and Neck Intergroup in their larynx-preservation randomized trial (RTOG 91-11)[24]. To determine the contributions of chemotherapy and radiotherapy to larynx-preservation the investigators conducted a randomized trial investigating three radiation-based treatments: induction cisplatin plus fluorouracil followed by radiotherapy, radiotherapy with concurrent administration of cisplatin, and radiotherapy alone. CCRT resulted in a significantly better laryngeal preservation and locoregional control than induction chemotherapy followed by radiotherapy or radiotherapy alone. Despite this better result in locoregional control and the decreased incidence of metastases in both chemotherapy-arms, no differences in survival were noticed between any of the arms. This was probably due to the confounding influence of salvage surgery which was performed most in the radiotherapy alone and induction chemoradiotherapy arms.

A comparable French randomized trial was performed in oropharyngeal cancer[25]. This study demonstrated an improved overall survival and locoregional control in the CCRT arm compared to radiotherapy alone. Differences in distant metastases were not observed. Some other trials including hypopharyngeal cancer reported similar results[26-29]. Most of these studies included anatomically unresectable advanced head and neck squamous cell carcinomas.

In resectable advanced head and neck squamous cell carcinoma, surgery and postoperative (or adjuvant) radiotherapy used to be standard care. A conceivable randomized trial determining differences in surgery and non-surgery approaches would be a study investigating surgery

and adjuvant radiotherapy versus CCRT. Such a study has been published only once[30]. One-hundred-nineteen patients were randomized and most of them had tumors of the oral cavity, oropharynx or larynx. No differences in disease free-survival or overall survival were noticed. The overall organ preservation rate was 45%, mostly including the laryngeal and hypopharyngeal site. Although, surgery remains an important modality, increasing evidence is emerging that CCRT improves organ preservation. Function is not always preserved and seems to be related to tumor size, treatment modality[9] as well as tumor location[31], no definitive answers are available to the question whether CCRT is superior to surgery plus radiotherapy in all advanced HNSCC's. Other new targeted therapy approaches are also upcoming combining "small molecules" or antibodies[32] with radiotherapy. Strong synergistic effects are observed of the IgG1 monoclonal antibody Cetuximab, which binds to the epidermal growth factor receptor, and radiotherapy[33]. Bonner et al.[34] reported in 2006 that the combination of cetuximab and radiotherapy appeared to be significantly more effective than radiotherapy alone (p= 0.005). The median duration of locoregional control was 24 months among patients treated with cetuximab plus radiotherapy (N=211) and 15 months among those given radiotherapy alone (N=213). A number of other molecular agents inhibiting transmembrane receptor tyrosine kinase are currently in various stages of development for clinical use in head and neck cancer[35,36].

Treatment: RADPLAT

Chemotherapy can be administered both intravenously and intra-arterially. Intra-arterial infusion used to be problematic because many complications occurred with direct insertion of a catheter into the external carotid artery. Nowadays, microcatheters can be used and permit superselective placement in small arteries thereby minimizing gravity and number of complications. Intra-arterial infusion of cisplatin makes it possible to deliver much higher doses, since the drug delivery takes place selectively into the nutrient artery and tumor bed. This allows for a first-pass effect of the drug. After the first passage, cisplatin will enter the systemic circulation. To neutralize the systemic toxic activity of this high dose cisplatin, sodium thiosulphate is administered intravenously. Sodium thiosulphate binds covalently to cisplatin forming a soluble complex. When the binding takes place in the plasma, the toxic effect of cisplatin is reduced. This has been described as plasma "clearance" of cisplatin[37]. The direct plasma "clearance" and the increased dose of cisplatin to the tumor are the therapeutic advantages of intra-arterial infusion of chemotherapeutical drugs[38]. This means that optimal therapeutic advantage of intra-arterial infusions (compared to intravenous infusion) can be obtained when the blood flow through the tumor is slow and cisplatin is cleared fast from the circulation[39].

Robbins et al.[39,40] were the first to investigate which dose of cisplatin was most successful in terms of a minimal systemic toxic effect and a favorable locoregional response rate. In 1992 they reported the first results of intra-arterial infusion of 150 mg/m^2 cisplatin in HNSCC patients undergoing subsequent surgery or radiotherapy. Patients with advanced and recurrent squamous cell carcinoma or sarcoma were included and an overall response

rate of 90% was achieved. After this initial remarkably positive result in recurrent disease, intra-arterial infusion of cisplatin ('PLAT') was combined with concurrent radiotherapy ('RAD', acronym: RADPLAT) with simultaneous intravenous infusion of sodium thiosulphate (9 mg/m^2). Four cycles of cisplatin (150 mg/m^2) were administered via a microcatheter, angiographically and superselectively placed in the tumor's dominant supply artery by an interventional radiologist after transcutaneous insertion into the femoral artery (Seldinger technique). In 2000, the first results of large series of 213 RADPLAT patients were reported by Robbins et al.[41]. Patients with advanced head and neck squamous cell carcinoma, mostly stage IV disease, had a 5-year locoregional control and overall survival of 74% and 39%, respectively. Similar results were presented by Balm et al.[42] and Homma et al.[43]. In these series the following surgical and functional unresectability criteria were applied: (1) oral cavity and base of tongue: no functional reconstruction possible after removal of the tumor, mainly including tumors requiring total glossectomy or resection of both hypoglossal nerves (2) tonsil and soft palate: extension towards the base of skull as manifested by clinical trismus and apparent on imaging, making it highly unlikely to obtain clear surgical margins at the cranial border (3) posterior pharyngeal wall tumors or hypopharyngeal carcinomas: requiring total laryngectomy and extensive reconstruction, or fixation to the cervical spine (4) supraglottic larynx and/or base of tongue: tumor extensions requiring total glossectomy and total laryngectomy for complete removal.

These reports indicate that RADPLAT treatment provides a good locoregional response, which is at least comparable with intravenous CCRT[44]. However, approximately 25% of all patients will still experience treatment failure or serious toxicity, which makes identification of predictive factors essential.

Toxicity

Chemoradiation treatment is frequently associated with serious toxicity and treatment interruptions. The synergistic effect of chemotherapy to radiotherapy causes more serious toxicity than radiotherapy alone. Severe (grade 3-4) mucositis has been reported in approximately 50 % of chemoradiation cases and acute toxicities like nausea/vomiting, leucopenia, anemia, renal dysfunction and dermatitis occur frequently as well. Late toxicity consists mainly of various degrees of swallowing problems, trismus, non-healing ulcers, osteoradionecrosis or loss of teeth and xerostomia, but detailed studies reporting late toxicity with a long follow-up remain limited. Because intra-arterial chemoradiation delivers a higher dose of cisplatin at the local site and cisplatin is mainly neutralized by sodium thiosulfate in the systemic circulation, one may expect higher toxicity in the head and neck region and decreased systemic toxicity. These toxicities are investigated and discussed later in this thesis. Furthermore, predicting toxicity and functional impairment is very difficult, but essential in pre-treatment clinical decision making. Serious toxicity may lead to decreased treatment compliance and in patients who will have residual disease, a toxic treatment can be spared. In those patients, alternative or palliative treatment can be offered. Consequently, pretreatment prognostic analysis may lead to better treatment results.

Survival analysis

"It is the best thing, in my opinion, for the physician to apply himself diligently to the art of forthknowing" - Hippocrates -. Hippocratic prognostication, however, differed considerably mainly from current prognostication in that the prognosis was inferred directly from the symptoms without passing through the process of diagnosis[45]. These days, prognosis is the knowledge of outcome and is based upon an accurate diagnosis, knowledge of the natural history of the disease, the disease's response to treatment, and the progression of the disease in the individual patient (Bailey, Concise Dictionary of Medical-Legal Terms) with implementation of pretreatment performance status and weight loss still being important prognostic factors. A particular cancer patient wants to know his or her prognosis based on all relevant factors rather than the overall probability of surviving for 5 years from limited survival statistics based on staging only. Besides individual prognosis, pretreatment knowledge about the expected toxicities comes also within reach. To address these issues, analysis of clinical findings, environmental factors and characteristics of patients are vital. Furthermore, prognostication contributes to efficient and good medical practice and it helps us to learn from our experience.

Time event outcomes are frequently used for prognostication. The time variable is determined by the interval between two dates during the clinical course and the event refers to an endpoint like recurrence, death or toxicity. Most studies in this thesis have looked at local control and overall survival as time-event outcome. Since intra-arterial chemoradiation treatment is a locoregional treatment, local control and regional control classify the success rate of this therapy best. Considering both outcomes, patients who had a local recurrence or regional lymph node recurrence are considered as treatment failures. Locoregional disease control as outcome requires adequate follow-up, because time to detection of the recurrence is essential. All studies described in this thesis were part of a phase 2 or 3 trial. Patients participating in the trials were evaluated 6 to 8 weeks after treatment and every 3 to 4 months after posttreatment evaluation in the first two years thereafter. When considering overall survival as outcome, death of any cause is considered as an event. Because several patients undergoing chemoradiation often have severe co-morbidity, and chemoradiation may be a potentially lethal treatment, registration of time of death is essential. If for example a patient was cured by chemoradiation but the patient dies a year later due to a cisplatin induced renal failure, treatment for this particular patient should be considered as a failure. Therefore overall survival statistics are an essential part of the prognostic analysis.

Both outcomes, overall survival and locoregional control, can be related to potential prognostic variables. A variable has prognostic value if it is able to demonstrate differences in outcome in two or more subgroups. Univariable analyses can be performed to identify potential prognostic factors and are also useful to reduce the number of potential prognostic factors. Kaplan-Meier curves[46] can show the differences in outcome between subgroups and the log-rank test will provide a *p*-value indicating if there is a significant difference. Some variables may have a strong intervariable relationship and this might be confounding. Confounding variables occur when one variable is related to outcome due to the fact that

another interrelated variable is strongly related to outcome. A multivariable analysis can be performed to detect confounding explanatory factors and to analyze the joined effect of both factors. A Cox proportional hazards analysis is an example of a multivariable analysis[47]. In a Cox model a stepwise weighing of interrelated factors is performed by canceling out the confounding effects of other prognostic factors. A Cox analysis builds a model consisting of only variables that have predictive value after adjustment for each other.

To illustrate results from the multivariable analysis, a nomogram can be used. The strength of a nomogram is the ability to illustrate the wide range of outcomes in a heterogeneous patient group. Nomograms may serve as a basis for more appropriate selection of patients, who benefit most from a treatment, such as targeted chemoradiation. It is also helpful in the selection of patients with a low probability of local control who may then become eligible for more aggressive or alternative treatment schedules. In the majority of the described studies in this thesis, these statistical tests have been applied to determine the prognostic accuracy of several potential prognostic factors for locoregional control or overall survival.

Predictive and prognostic factors

Predictive factors provide information about the outcome of patients after a specific treatment, whereas prognostic factors are associated with outcome independent of treatment. Predictive factors are associated with locoregional control and prognostic factors are more related to survival.

Host-related factors typify the patient and are not directly related to the tumor and usually unique to the patient. These factors generally include demographical characteristics (race, age and gender), co-morbidity and nutritional status. Age has proven to be an important potential prognostic factor for survival. Lacy et al.[48] found in a cohort of 1030 patients with HNSCC that patients under 40 have a better prognosis than older people. However, one should realize that for overall survival, age is a prognostic factor in all diseases. Gender is another important factor: male patients generally do worse than female patients. For example, Faye-Lund et al.[49] investigated 500 patients with HNSCC. Female patients had a significantly better overall survival than male patients and gender was an independent prognostic factor. However, age and gender are often found to be confounding factors, requiring a multivariable analysis in studies investigating potential prognostic factors.

HNSCC patients usually have a long history of chronic alcohol consumption and tobacco abuse playing a pivotal role in the development of squamous cell carcinoma[50]. In general, these habits go along with poor nutritional status and co-morbidities. Patients often present with weight loss and low hemoglobin levels[51]. Both nutritional status and performance status are important factors for predicting outcome[52,53]. Co-morbidity is particularly important in case of toxic or intensive therapies and may even determine whether or not treatment should be continued. Several investigators have developed co-morbidity scores. An example of this is Adult Co-morbidity Evaluation-27 (ACE-27). The ACE-27 is a validated tool which classifies specific diseases into 4 subgroups: severe, moderate, mild or no co-morbidity. Co-morbidity has been shown to be prognostic for survival in several studies[53-55].

Whether patient-related factors are important in predicting outcome in intra-arterial CCRT will be discussed in chapter 2.

Apart from general patients' characteristics, prognosis is also linked to tumor characteristics. Tumor characteristics include size and site of the tumor, tumors stage, histological as well as genetic factors. TNM-staging system determines the size of the primary tumor, nodal and distant metastasis. It has been developed more than 50 years ago and is -after several updates- still the most used staging system in the world. Although the TNM staging is able to distinguish outcomes of patients with small and large tumors, it has limited value for prognostic differentiation of patients with advanced tumors[56]. Whereas the TNM system is very useful for planning of surgery, tumor volume might be more relevant for radiotherapy. Tumor volume measurements are time consuming and probably therefore not often used in routine practice. However, if used as variable in prognostic analyses, tumor volume was often identified as an independent prognostic factor[57-62]. Due to the wide range of measured volumes per site in literature, data are difficult to compare.

Tumor volumes are often determined with help of computer tomography (CT) or magnetic resonance imaging (MRI). Both CT and MRI can be used for prognostic volume measurements in HNSCC[57,62]. The value of PET-CT remains to be determined with respect to volume assessment[63] and it is not unlikely that standard uptake value (SUV)[64] will also develop into a prognostic factor for outcome after head and neck cancer treatment.

Apart from tumor stage, site and volume, investigators have studied the correlation between biological markers and outcome. As many cellular processes are involved in resistance to radiation or chemotherapy, such markers include those associated with cell cycle control (cyclin D1, retinoblastoma gene product (RB, p16, p21 and p27), apoptosis (TP53, TP63, TP73, murine double minute 2 (MDM2), bax, bcl-2 and bcl-xl), growth regulation (epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), PCNA and ki-67), angiogenesis (VEGF and FGF), focal adhesion signaling (cortactin), hypoxia (carbonic anhydrase IX (CA9) and hypoxia-inducible factor 1αlpha (HIF-1α)),sensitivity to chemotherapy (XPA, multidrug resistance-associated protein 2 (MRP2) and P-glycoprotein), and probably many others[65-74]. A general conclusion from all these studies is that many mechanisms can influence resistance to radiotherapy or cisplatin and thus more than one marker will be needed to assess an individual patient's treatment response. Tumor marker profiles in HNSCC patients may be more valuable if they include at least several markers with unrelated or mutually opposing biologic roles so that statistical assessments in combination may divide patients into meaningful therapeutic groups. Recently, advances in molecular diagnostics like development of microarrays, tissue microarrays (TMA) and comparative genomic hybridization (CGH), have made it possible to study genetic abnormalities and gene expression changes in many tumors and relate these to outcome measures. Ideally, analysis of pre-treatment biopsies should make it possible to differentiate patients into treatment-resistant and treatment-sensitive groups. In chapter 3 and 4 it is shown that these analyses can distinguish between chemoradiation-sensitive patients and chemoradiation-resistant patients.

Aim and brief outline of thesis

The main goal of this thesis is to investigate the role of several potential predictive factors for outcome in patients with HNSCC treated with concurrent intra-arterial chemoradiation. We were interested in the following outcomes: local control, regional control, overall survival and toxicity. The role of pretreatment clinical factors like tumor-related factors is demonstrated in chapter 2, whereas chapter 5 describes post-treatment factors. The influence of genetic factors on local control and survival is reported in chapter 3 and 4. Chapter 6 focuses on predictive factors for regional control. The role of salvage neck dissections after chemoradiation is discussed as well in this chapter. Chapter 7 elucidates the association between several factors and toxicity.

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CHAPTER 2

Pretreatment probability model for predicting outcome after targeted chemoradiation

Guido B. van den Broek, Coen R.N. Rasch, Frank A. Pameijer, Ellen Peter, Michiel W.M. van den Brekel, I. Bing Tan, Jan H. Schornagel, Josien A. de Bois, Lambert J. Zijp, Alfons J.M. Balm

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ABSTRACT

BACKGROUND. Concurrent chemoradiation is increasingly used in patients with advanced head and neck cancer. A clinical nomogram was developed to predict local control and overall survival in individual patients, who will undergo chemoradiation.

METHODS. Ninety-two consecutive patients with stage III and IV squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx and supraglottic larynx were treated with selective-targeted chemoradiation (acronym: "RADPLAT"); all living patients had a minimum follow-up of 2 years. Besides general factors, the following parameters were analyzed in a multivariable analysis: primary tumor volume, nodal tumor volume, total tumor volume, lowest involved neck level, co morbidity, pretreatment hemoglobin, pretreatment weight loss and uni-/bilateral intra-arterial infusion. Relevant factors for local control and survival were analyzed using the Cox proportional hazards model.

RESULTS. At 5 years: local control and overall survival for the whole group were 60% and 38%, respectively. Primary tumor volume (hazard ratio, 1.03; p= 0.01) and unilateral infusion (hazard ratio, 5.05; p= 0.004) influenced local control significantly. Using tumor volume as a continuous variable an adjusted risk ratio of 1.026, was found, indicating that each 1 cm^3 increase in volume was associated with 2.6 % decrease in probability of local control. Primary tumor volume (hazard ratio, 1.01; p= 0.003), co morbidity (ASA physical status 1 vs. > 1; hazard ratio, 2.47; p= 0.01), lowest involved neck level (hazard ratio, 3.45; p= 0.007) and pretreatment weight loss > 10% (hazard ratio, 2.04; p= 0.02) were significant predictors for worse overall survival. Variables from the multivariable analysis were used to develop a nomogram capable of predicting local control and overall survival.

CONCLUSIONS. Tumor volume plays a significant role in predicting local control and overall survival in advanced head and neck cancer patients treated with targeted chemoradiation. The developed nomograms may be useful for pretreatment selection of patients with advanced head and neck cancer.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common cancer in men with a worldwide incidence of approximately 780 000 new cases per year[1]. Over 70% of head and neck cancer patients present with advanced stage III and IV disease. Treatment for patients with advanced, inoperable HNSCC is a therapeutic challenge. There is increasing evidence that concomitant chemoradiation leads to improved local control and overall survival in advanced head and neck cancer, as compared with conventional radiotherapy. This makes this treatment modality more suitable as curative treatment option in these patients[2-8]. Clinical trials comparing different treatment schedules (e.g. differences in administration route (intra-arterial vs. intravenous), chemotherapy dose and radiation schedules) are ongoing to optimize the concomitant delivery of chemotherapy and radiation. However, chemoradiation is frequently associated with serious toxicity[3,4]. Patients unlikely to be cured with this chemoradiation should ideally be recognized before treatment. Assessment of factors that significantly influence local control and survival is therefore essential. There are a number of recognized prognostic factors for outcome including gender, hemoglobin level[9-11], co morbidity[12] and tumor volume[13]. Information on the prognostic value of these factors in chemoradiation remains scarce. Unfortunately, powerful predictors used in surgical patients i.e. depth of tumor invasion[14], number of positive lymph nodes and extracapsular spread[15,16] are not accessible for patients treated with primary chemoradiation.

In this study we investigated the role of aforementioned prognostic factors in predicting local control (LC) and overall survival (OS) after targeted chemoradiation in patients with advanced head and neck cancer. To assess the simultaneous effect of various factors on predicting LC/OS, a logistic regression model was used. By combining the significant prognostic factors we developed a clinical algorithm for LC and OS to facilitate clinical decision-making.

PATIENTS AND METHODS

Between April 1997 and May 2001, 105 consecutive patients with newly diagnosed inoperable T3-T4 squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx and supraglottic larynx were enrolled for targeted chemoradiation. Except for hypopharyngeal carcinoma, all tumors were classified as surgically or functionally inoperable. Inclusion criteria were as follows: (1) oral cavity and base of tongue: no functional reconstruction possible after removal of the tumor, mainly including tumors requiring total glossectomy or resection of both hypoglossal nerves (2) tonsil and soft palate: extension towards the base of skull as manifested by clinical trismus and apparent on imaging, making it highly unlikely to obtain clear surgical margins at the cranial border or requiring resection of the whole soft palate (3) posterior pharyngeal wall tumors or hypopharyngeal carcinomas: requiring total laryngectomy and extensive reconstruction, or fixation to the cervical spine (4) supraglottic larynx and/or base of tongue: tumor extensions requiring total glossectomy and total laryngectomy for

complete removal. All statements regarding unresectability were reviewed by the three head and neck surgeons involved in this study (A.J.M.B., M.W.M.v.d.B., I.B.T.).

For this study, three patients were excluded because distant metastases were detected just before start of the treatment. Another 10 patients were excluded because good quality pretreatment MR imaging was not available for tumor volume measurements, resulting in a study population of 92 patients. All living patients had minimal 2-years follow-up. This study population included 69 men and 23 women with a median age of 53 years (range 29-78). Tumors were staged according to the UICC guidelines[17]. The T and N-classification distribution was as follows: T3 (22), T4 (70), N0 (33), N1 (6), N2a (1), N2b (18), N2c (26), N3 (8), resulting in 13 patients with stage III and 79 patients with stage IV disease. The following site distribution was established: oral cavity (n= 22), oropharynx (n= 58), hypopharynx (n= 9) and supraglottic larynx (n= 3). Patient, tumor and treatment characteristics are summarized in Table 1 and 2.

Targeted chemoradiation has been described earlier[18,19]. Briefly, treatment consisted of four consecutive weekly selective intra-arterial infusions of cisplatin (150 mg/m^2) simultaneous with intravenous sodium thiosulfate rescue combined with radiotherapy (2 Gy per day, 5/week x 7 to a total dose of 70 Gy) according to the RADPLAT protocol[20]. In addition to the earlier reported intra-arterial administration of cisplatin[21], we performed bilateral infusion in patients whose primary tumors extended across the midline, with equal distribution of cisplatin doses over both sides. Before the start of treatment, all patients signed an informed consent form approved by our institutional protocol review committee.

Tumor volume assessment was performed by delineation of all visible tumor tissue on pretreatment MR imaging. MRI examinations were performed on a 1.5-Tesla scanner (Siemens Magnetom 63 SP4000; Siemens, Erlangen, Germany). Slice thickness was 4 mm or less, with interslice gap of 1 mm or less. The field of view for the axial views was 16 to 18 cm for T1-weighted sequences and 18 to 20 cm for T2-weighted sequences. T1-weighted images were obtained before and after injection of intravenous gadolinium. Post-contrast images were acquired using fat-saturation. An experienced head and neck radiologist (F.A.P.), who was blinded to patient's outcome, performed primary tumor volume delineations. Delineations as performed by the radiologist were then transferred into a computer with digitized MR images and delineation tools (G.v.d.B.). Twenty-nine good quality MRI scans from referral hospitals were redigitized for tumor volume measurements. Primary tumor volume data are shown in Figure 1. Volumes of lymph node metastases were calculated by the summation of all pathological nodal tissue on MRI (G.v.d.B.). To minimize the risk of measuring reactive lymph nodes, only lymph nodes fulfilling following criteria were delineated: a) shortest diameter ≥ 15 mm, b) signs of central necrosis or c) confirmation of malignancy by (ultrasound-guided) fine needle aspiration cytology.

Patient-related factors were gender, age, pretreatment hemoglobin level, pretreatment weight loss (% of body weight) and co morbidity (ASA physical status; always assesses before pretreatment examination under general anesthesia by the attending anesthesist). Tumor-related factors were T-classification, N-classification, TNM-stage, primary tumor

TABLE 1 Patient population

Variable	N	(%)
Gender	69	75
Male	23	25
T- classification		
T3	22	24
T4	70	76
N- classification		
N0-N1	39	42
N2-N3	53	58
TNM-stage		
Stage III	13	14
Stage IV	79	86
Site		
Oral cavity	22	24
Oropharynx	58	63
Supraglottic larynx	3	3
Hypopharynx	9	10
Neck level involved		
No, Level II - III	83	90
Level IV	9	10
Pretreatment weight loss		
< 10 %	47	51
≥ 10 %	45	49
Co morbidity (ASA)		
1	26	28
2-3	66	72
Infusion mode		
Unilateral	40	46
Bilateral	46	54

TABLE 2 Continuous variables

Variable	median	minimum	maximum
Age (years)	53	29	78
Hemoglobin level (mmol/l)	8.5	6.2	10.7
Primary tumor volume (cm3)	35.4	6.4	393.0
Nodal tumor volume (cm3)	18.1	2.3	131.7
Total tumor volume (cm3)	42.5	6.9	393.0

volume, nodal tumor volume, total tumor volume, tumor site and neck level involvement. The treatment-related factor was unilateral or bilateral intra-arterial infusion of cisplatin.

Treatment response was evaluated six to eight weeks after completion of radiotherapy by MRI, followed by examination under general anesthesia. If the primary tumor site was

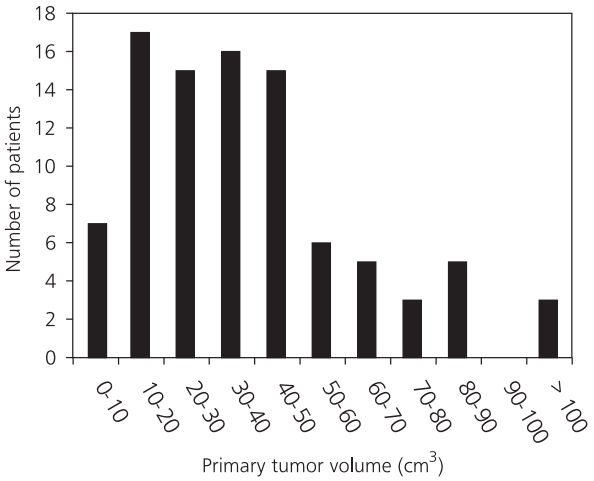

FIGURE 1. Distribution of primary tumor volumes (cm^3) for the patient population.

macroscopically suspect for residual tumor, a biopsy was taken during examination under general anesthesia. Thereafter, patients were subjected to regular outpatient follow-up and an annual chest X-ray.

Statistical methods

Differences in means were compared using the Student *t* test. Univariable (not shown) and multivariable analyses were performed to assess the effects of various factors on predicting outcome (local/regional control and overall survival). Cox proportional hazards model[22] was used to perform the multivariable analysis. Six of the 92 patients could not be assessed for local control after completion of treatment due to death of following causes: pneumonia (n = 3), cervical spondylitis (n = 1), rupture abdominal aneurysm (n = 1) and arterial (carotid artery) bleeding (n = 1). These patients were not excluded from survival analysis. Continuous variables (e.g. tumor volumes, hemoglobin level and age) were tested on linearity and time dependency. The final multivariable analysis was adjusted for age, because age demonstrated no linear association with local control and overall survival. In case of interactions, the variable with the most influence in the multivariable analysis was chosen to enter the final multivariable model. Besides the aforementioned criterion, all variables were entered at the multivariable phase, regardless the outcome of the univariable analysis. The forward stepwise selection procedure in which non-significant variables from the univariable analysis are not reanalysed in the multivariable analysis may be inferior for maximizing prognostic accuracy[23], and was not preferred. The final multivariable model was generated by a backward elimination method to determine factors with influence on outcome. Because T- and N-classification are generally used prognostic variables, they were included in all final multivariable analyses. The procedure PROC PHREG (SAS system for Windows release 8.02) was used to perform the multivariable analyses.

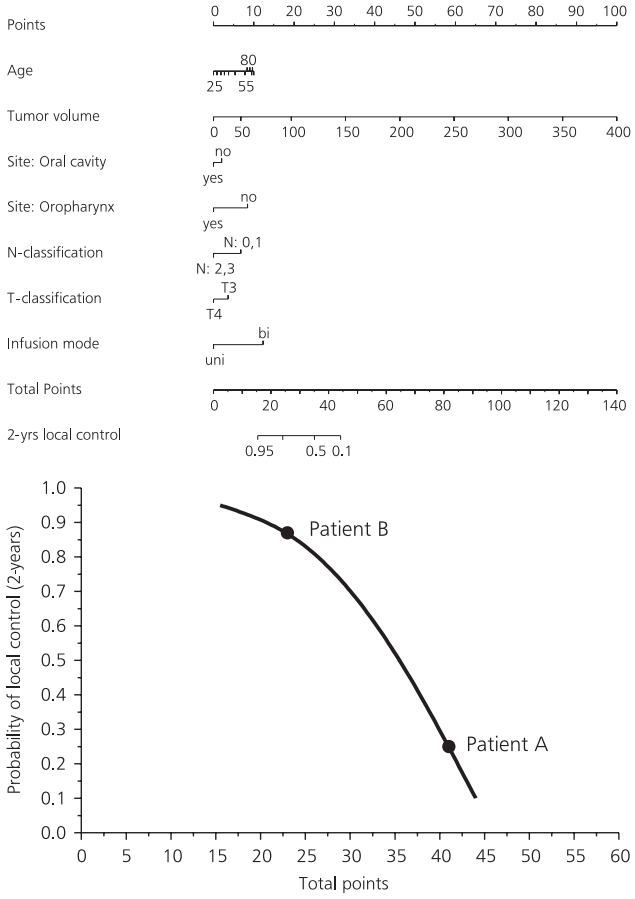

FIGURE 2. Clinical algorithm based on 86 RADPLAT patients for predicting the probability of 2-year local control. (A) Nomogram. (B) Translation graph. Information for use: first, identify patient's score for each variable listed in the nomogram. Second, add all individual variable scores to form a total. The total score determines a certain location on the 'total points' axis. This location defines the probability of local control on the 'probability' axis. For example, it is shown that a 64-year (10 points) old patient A with a T4N2 (0 points) tumor (primary tumor volume of 56 cm^3, 9 points) of the oral cavity (9 points), who is treated by bilateral intra-arterial infusion (13 points) has a much lower probability of local control (total 41 points, probability of local control: 25%) than a 72-year (10 points) old patient B with a T4N1 (8 points) tumor (primary tumor volume of 20 cm^3, 3 points) of the oropharynx (2 points), who is treated by unilateral intra-arterial infusions (0 points, total 23 points, probability of local control: 87%).

Calculated coefficients from the final multivariable model were converted into a 0-100 scale. The maximum score (100) was based on the maximum coefficient. All coefficients for each prognostic group were then plotted relative to this maximum (Figure 2A and 3A). Summation of each variable score resulted in an overall score (total points). The total points were finally converted into a probability of local control/overall survival (Figure 2B and 3B). This allowed summation of the risks for any combination of prognostic variables.

RESULTS

Tumor volume

Primary tumor volumes ranged from 6.4 to 393.0 cm^3 (Table 2, Figure 1). Median primary tumor volumes stratified by site were as follows: oral cavity, 39 cm^3; oropharynx, 37 cm^3; hypopharynx, 32 cm^3 and supraglottic larynx, 19 cm^3. Patients with clinical evidence of nodal disease had nodal tumor volumes ranging from 2.3 to 131.7 cm^3. Nodal tumor volume was associated with N-classification. Patients with N3 disease (median 75.3 cm^3) had bigger nodal

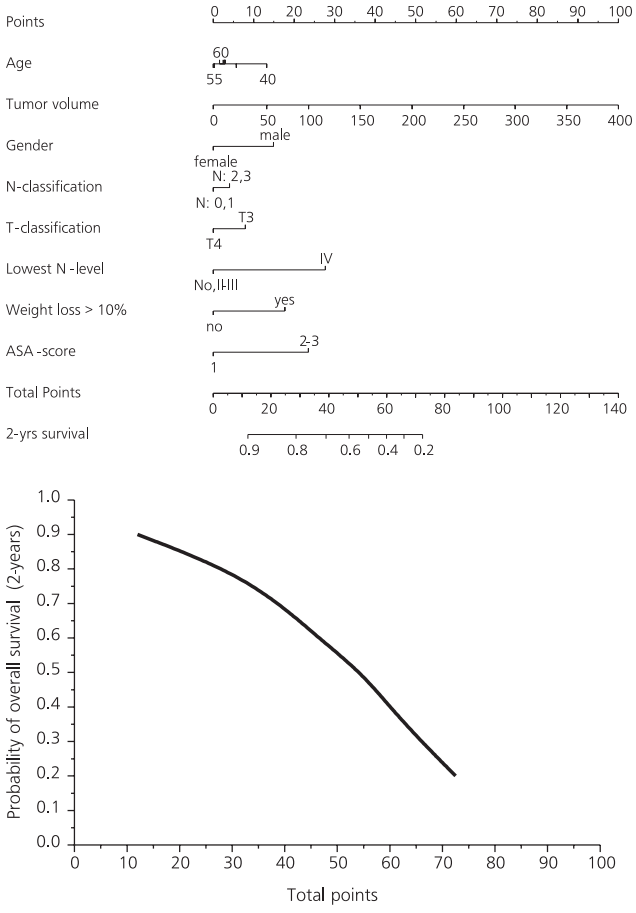

FIGURE 3. Clinical algorithm based on 92 RADPLAT patients for predicting the probability of 2-year overall survival. (A) Nomogram. (B) Translation graph. Information for use: first, identify patient's score for each variable listed in the nomogram. Second, add all individual variable scores to form a total. The total score determines a certain location on the 'total points' axis. This location defines the probability of overall survival on the 'probability' axis.

tumor volumes than N1 (median 0.7 cm^3) and N2 (median 11.1 cm^3) patients. Total tumor volumes ranged from 6.9 to 393.0 cm^3 (mean 57.2 cm^3, median 42.5 cm^3). Mean primary tumor volumes of unilateral and bilateral intra-arterial infused patients were similar and not significant different (p= 0.94), 40.5 cm^3 and 39.8 cm^3 respectively.

Local and regional control

A complete local response after 6-8 weeks was achieved in 79 (92%) and a partial response in 7 (8%) patients. After 2 years, 25 patients had a local failure, including all partial responders. Six of them underwent salvage surgery with 3 incomplete resections and only 2 of these 6 patients survived longer than 7 months. Sixty-one patients had local control of their disease at the primary site. For all patients, the estimated local control rate at 5 years was 60%. Seven (8%) patients had a regional failure. Regional failure was defined as persistent disease following chemoradiation or regional recurrence without local failure. The low number of regional failures made analysis of prediction for regional control unreliable and was therefore not performed.

Multivariable analysis was used to determine the association between factors and local control. Besides T- and N-classification, primary tumor volume, age, tumor site and uni-/bilateral intra-arterial infusion were included in the final multivariable analysis for local control. After this final analysis, two factors had significant predictive value for local control: primary tumor volume (p= 0.01) and unilateral intra-arterial infusion (p= 0.004, Table 3). Using tumor volume as a continuous variable an adjusted risk ratio of 1.026, was found, indicating that each 1 cm^3 increase in volume was associated with 2.6 % decrease in probability of local control.

A nomogram (Figure 2A) was constructed based on factors used in the final multivariable analysis. The nomogram gives a total score that can be translated to a probability of local control (Figure 2B). Patients with a total score of 60 will have the worst prognosis. An example is set in figure 2.

Overall survival

After a median follow-up of 35 months (range 25-78 months), 37 patients remained alive. Thirty-two patients died of tumor-related causes, 8 patients of a second primary cancer, 7 patients of other causes (CVA (n= 2), pneumonia (n= 3), lung embolus (n= 1), myocardial infarction (n= 1)) and 1 died of an unknown cause. This patient died 18 months after start of the treatment and was physically examined 6 weeks before death in the outpatient clinic without evidence of recurrent disease. Autopsy was not performed. Only one patient was lost to follow-up. The estimated 5-year overall survival rate for the whole study group, including the six patients who died during treatment, was 38%.

TABLE 3 Cox proportional hazards analysis for local control

Variable	p- value	Hazard Ratio	95% Hazard Ratio Confidence Limits
Site (oral cavity vs. other sites)	0.71	0.76	0.17 - 3.42
Site (other sites vs. oropharynx)	0.13	0.33	0.08 - 1.39
T- classification (T3 vs. T4)	0.36	0.58	0.18 - 1.87
N- classification (N0-1 vs. N2-3)	0.07	0.42	0.16 - 1.07
Primary tumor volume	0.01	1.03	1.01 - 1.05
Infusion mode (uni- vs. bilateral)	0.004	5.05	1.69 - 15.08

TABLE 4 Cox proportional hazards analysis for overall survival

Variable	p- value	Hazard Ratio	95% Hazard Ratio Confidence Limits
N- classification (N0-1 vs. N2-3)	0.66	1.16	0.61 - 2.21
T- classification (T3 vs. T4)	0.40	0.72	0.34 - 1.55
Gender (male vs. female)	0.07	0.49	0.23 - 1.05
Weight loss (<10 % vs. ≥10%)	0.02	2.04	1.10 - 3.78
Co morbidity (ASA 1 vs. ASA 2-3)	0.01	2.47	1.21 - 5.04
Neck level (No, Level II-III vs. IV)	0.007	3.45	1.40 - 8.48
Primary tumor volume	0.003	1.01	1.004 - 1.017

Besides T- and N-classification, primary tumor volume, gender, age, pretreatment weight loss, neck node level involvement and co morbidity were included in the final multivariable analysis for overall survival. This analysis demonstrated that primary tumor volume (p= 0.003), neck node level involvement (p= 0.007), co morbidity (p= 0.01) and pretreatment weight loss (p= 0.02) were independent prognostic factors for overall survival (Table 4). Based on variables in the final multivariable analysis, a nomogram for predicting probability of 2-year overall survival was constructed (Figure 3A and 3B). This nomogram visualizes the importance of above-mentioned prognostic factors for overall survival. In the nomogram age below 40 years was not shown, because only 4 patients were younger than 40 years and in this series all had a bad outcome and probably do not give a good representation of this subgroup. However, to exclude biased data we did include these four patients in the multivariable analysis.

DISCUSSION

Our estimated 5-year local control and overall survival rates were 60% and 38%, respectively, which are in agreement with the reported local control and survival data of Robbins et al.[19]. In developing a prognostic model we were able to confirm several previously described prognostic factors like primary tumor volume[24] and co morbidity[12]. In addition, we found a predictive factor (unilateral (versus bilateral) intra-arterial infusion of chemotherapy) for local control, which has not been described before.

Primary tumor volume emerged as an independent significant factor for predicting local control and overall survival. This confirms earlier published results of many studies on patients treated with radiotherapy alone or with chemoradiation[13,24-36]. An overview of literature (Table 5) demonstrates a variety of mean tumor volumes and site-dependent cut-off volumes. Patients with comparable primary tumor volumes of different sites seem to have different local control probabilities. For a certain volume of a laryngeal tumor, the probability of local control is lower than for a hypopharyngeal tumor with the same volume[13]. These differences were also found when we compared hypopharyngeal and oropharyngeal carcinomas. Nathu et al.[26] presented a 5-year local control rate of 86% in a group of 35 oropharyngeal cancer patients with a mean tumor volume of 14.8 cm^3, whereas Hermans et al.[27] presented a 5-year local control rate of 75% in a group of 119 laryngeal cancer patients with a mean tumor volume of 2.3 cm^3. Some authors used cut-off volumes to separate patients in favorable and unfavorable groups. Cut-off volumes have limited value for clinical use and linear correlations as found in glottic and supraglottic laryngeal cancer[27,28,32] seem to be more practical for individual use. We could demonstrate a near linear correlation in our material using volume as a continuous variable in the multivariable analysis: 1 cm^3 rise of tumor volume resulted in 2.6% decrease of local control (p= 0.01). This finding enables us to implement volume measurement as a tool for clinical decision-making.

The effectiveness of concomitant chemoradiation is also suggested by comparison of tumor volumes between treatment modalities (Table 5). Hermans et al.[25] presented a 5-year local control rate of 47% in oropharyngeal cancer patients with a mean tumor volume of 15

TABLE 5 Overview of literature: primary tumor volume and local control rates for different head and neck sites after radiotherapy (RT) or concomitant chemoradiation (CCRT)*

Authors	RT/ CCRT	Site	N	Volume (mean, cm^3)	Local control (5-year)	Comment
Hermans et al.[25]	RT	oropharynx	9	3(T1)	80**	Only tonsilcarcinoma.
			28	11(T2)	60	
			33	15(T3)	47	
			42	45(T4)	37**	
Nathu et al.[26]	RT	oropharynx	49	7(T2)	92	
			35	15(T3)	86	
			16	43(T4)	75	
Mendenhall et al.[13]	RT	oropharynx (ts)	69	18	86	
		oropharynx (bot)	72	24	84	
		oropharynx (sp)	37	12	74	
		hypopharynx	45	6	85	
		supraglottic larynx	114	8	76	
		glottic larynx	55	3	68	
Hermans et al.[27]	RT	glottic larynx	119	2.3	75	
Hermans et al.[28]	RT	supraglottic larynx	103	10.9	62	
Castelijns et al.[29]	RT	larynx	80	2.93	62	supraglottic (N=21), glottic (N=57), subglottic (N=2)
Mendenhall et al.[30]	RT	glottic larynx	37	≤ 3.5***	87	
				> 3.5***	29	
Freeman et al.[31]	RT	supraglottic larynx	31	< 6***	83	
				≥ 6***	46	
Mancuso et al.[32]	RT	supraglottic larynx	63	< 6***	89	
				≥ 6***	52	
Pameijer et al.[33]	RT	glottic larynx	42	< 3.5***	85	Only T3
				≥ 3.5***	25	
Pameijer et al.[34]	RT	hypopharynx	19	< 6.5***	89**	
			4	≥ 6.5***	25**	
Doweck et al.[24]	CCRT	oropharynx	23	18	83	
		hypopharynx	19	21	84	
		supraglottic larynx	7	13	100	
		glottic larynx	4	4.6	75	
this series	CCRT	oropharynx	55	37	76	
		hypopharynx	8	32	63	
		oral cavity	21	55	57	

*Intra-arterial chemoradiation
**2-year
***cut off volumes instead of mean volumes were used
ts: tonsil; bot: base of tongue; sp: soft palate

cm^3 treated with radiotherapy alone, whereas Doweck et al.[24] demonstrated a 5-year local control rate of 83% in oropharyngeal patients with a mean tumor volume of 18 cm^3 treated with chemoradiation.

Patients with tumor extensions across the midline were treated by bilateral intra-arterial infusions of cisplatin. However, bilateral intra-arterial infusions resulted in significantly more recurrences than unilateral intra-arterial infusions (p= 0.004). This is not explained by differences in tumor volumes between groups. A possible explanation could be that patients with tumor extensions across the midline are probably much better off with unilateral intra-arterial infusions following the neovasculature than with bilateral intra-arterial infusions distributing halved dosages over both sides. Future validation of this finding is needed with emphasis on arteriografic studies and intratumoral distribution of cisplatin.

After multivariable analysis, other clinical factors had no influence on local control. Even the often-found predictor of pretreatment hemoglobin level[9-11] did not emerge as significant for local control after concomitant chemoradiation. Many of our patients had blood transfusions during treatment, which might be an explanation for this outcome.

Primary tumor volume, lowest neck level involvement, pretreatment weight loss and co morbidity were identified as independent prognostic factors for overall survival. The inverse relationship between co morbidity and survival has already been established in a number of studies[12,37-39]. These studies demonstrated significant correlations between co morbidity and survival in young[12] and advanced[37] laryngeal cancer patients undergoing radiotherapy. Our data confirm that co morbidity has influence on overall survival in chemoradiation patients as well and makes pretreatment assessment of co morbid conditions a prerequisite. Nutritional status has not often been described in prognostic studies. In this journal, it has been reported that preoperative weight loss is a prognostic factor for worse survival[40] in male patients. Our data demonstrate that pretreatment weight loss has prognostic value in chemoradiation patients as well. We could not confirm the earlier described association between lower neck level involvements and distant metastasis[41,42], since in only 2 out of 9 (22%) patients with lower neck level (Level IV) involvement distant metastases were detected, compared to 17 of 83 (20%) patients without lower neck level involvement.

With use of all factors from the multivariable analysis, we constructed a nomogram. The strength of a nomogram is the ability to illustrate the wide range of outcomes in a heterogeneous head and neck cancer patient group. The nomograms for local control and overall survival may serve as a basis for more appropriate selection of patients, who benefit most from targeted chemoradiation. It is also helpful in the selection of patients with a low probability of local control who may then become eligible for more aggressive or alternative treatment schedules. However, this nomogram should preferably be validated in an independent patient cohort before it can be used in clinical practice.

In conclusion, we found that in patients with advanced, unresectable head and neck cancer treated with targeted chemoradiation primary tumor volume is the most important independent prognostic factor. With use of clinical algorithms pretreatment selection of advanced head and neck cancer patients can be improved.

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CHAPTER 3

Genetic abnormalities associated with chemoradiation resistance of head and neck squamous cell carcinoma

Guido B. van den Broek, Volkert B. Wreesmann, Michiel W.M. van den Brekel, Coen R.N. Rasch, Alfons J.M. Balm, Pulivarthi H. Rao

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ABSTRACT

BACKGROUND. To identify reliable predictors of chemoradiation resistance of advanced head and neck squamous cell carcinoma (HNSCC).

METHODS. We performed a matched-pair analysis of 20 chemoradiation-resistant and 20 sensitive HNSCC, identified among a series of 104 consecutively treated cases. We compared the global DNA copy number profiles derived from comparative genomic hybridization (CGH) analysis of both groups to identify genetic markers associated with chemoradiation resistance.

RESULTS. Although sensitive and resistant case groups were characterized by a similar total number of genetic aberrations, high level amplifications were more frequent in resistant tumors. Resistant tumors were characterized by a different profile of genetic changes. Gains of 3q11-q13, 3q21-q26.1 and 6q22-q27 and losses of 3p11-pter and 4p11-pter were significantly associated with chemoradiation resistance. High-level amplifications unique to resistant cases involved the chromosomal regions 1p32, 3q24, 7p11.1, 7p11.2-12, 8p11.1, 8p11.1-12, 12q15, 13q21, 15q12, 18p11.3 and 18q11.

CONCLUSIONS. Sensitive and resistant HNSCC are characterized by divergent genomic profiles. These profiles may be valuable as predictive markers of treatment failure.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is an aggressive disease with a large proportion of cases presenting with advanced (stage III/IV) disease[1]. Adequate management of advanced HNSCC requires an aggressive approach but is limited by the density of vital and functionally important structures in the head and neck region[2]. In recent years, the application of concurrent platinum-based chemoradiation has emerged as an attractive alternative to traditional surgical management of advanced HNSCC. For example, primary chemoradiation offers the potential for functional preservation without survival compromise in the setting of advanced laryngeal carcinoma[3] and other advanced head and neck cancers. The significance of concurrent chemoradiation is further exemplified by recent studies demonstrating survival benefit of concomitant chemoradiation over radiation therapy alone in the adjuvant setting or in case of unresectable HNSCC[4-8]. Although these studies clearly demonstrate the overall sensitivity of HNSCC to concurrent chemoradiation, a significant number of individual cases experience locoregional treatment failure. These patients suffer potential side effects and toxicities of chemoradiation (mucositis, xerostomia, swallowing problems, ototoxicity, renal and other toxicities) without benefit[9,10]. Clarification of the molecular basis of chemoradiation resistance is needed to reveal reliable predictors of treatment failure and provide clues for the development of novel therapeutic approaches aimed at modulation of chemoradiation resistance.

Resistance of tumor cells to radiation and cisplatin is likely multifactorial. Recent studies have shown that abrogation of pro-apoptotic p53 signaling may aid cellular survival after cytotoxic stress[11]. Accordingly, chemoradiation resistant HNSCC show a high rate of p53 aberrations and increased expression of MDM2, a protein that shuttles p53 into degradative pathways[12,13]. In addition to p53 abrogation, several chromosomal alterations including amplifications of genes involved in detoxification of cytotoxic agents, deletion of genes involved in DNA repair, and various uncharacterized chromosomal alterations have been associated with chemotherapy and radiation therapy resistance of malignancies such as HNSCC[14]. For example, Akervall and colleagues[15] found a higher rate of chromosomal alterations in cisplatin resistant HNSCC cell lines relative to their sensitive counterparts. These data suggest that chromosomal instability and associated selection for chromosomal alterations may underlie adaptability of HNSCC to selection pressures such as chemoradiation treatment.

In order to explore the suggested chromosomal basis for chemoradiation resistance further, we performed a matched-pair comparative genomic hybridization analysis of chemoradiation sensitive and chemoradiation resistant HNSCC. Our data suggest that chemoradiation-resistant and -sensitive HNSCC are characterized by divergent chromosomal profiles, the significance of which remains to be determined.

PATIENTS AND METHODS

Patient population and tissue samples

One hundred and four previously untreated consecutive patients with advanced (stage III-IV) HNSCC (oral cavity, oropharynx, supraglottic larynx and hypopharynx) treated with the RADPLAT protocol[16] at the Netherlands Cancer Institute were the subjects of this study. Treatment consisted of four consecutive weekly selective intra-arterial infusions of cisplatin (150 mg/m2) simultaneous with intravenous sodium thiosulfate rescue combined with conventional radiotherapy (70 Gy) as described in detail elsewhere[17]. Treatment response was evaluated six to eight weeks after completion of radiotherapy by MRI, followed by examination under general anesthesia. Thereafter, patients were subjected to regular outpatient follow-up.

Twenty-six (25%) patients with histopathologically proven residual disease or recurrence after treatment were observed in the study group and deemed chemoradiation resistant. These were clinically matched with 26 patients without residual disease or recurrence after at least 2 years of follow up (chemoradiation sensitive). Matching criteria included tumor volume, TNM-stage, age, gender, anatomic location and infusion mode (uni- or bilateral). Archival paraffin-embedded pretreatment biopsies from chemoradiation sensitive and resistant primary tumors were histological confirmed to contain >70% of tumor tissue. DNA was extracted as previously described[18].

Comparative genomic hybridization (CGH)

CGH analysis was performed as described previously[19]. Briefly, equal amounts (2 microgram) of tumor DNA and normal human placenta DNA were labeled with fluorescein-12-dUTP (FITC) and Texas red-5-dUTP (Perkin Elmer, Boston, MA), respectively, co precipitated with 15 microgram of human cot-1 DNA (Invitrogen, Carlsbad, CA), and suspended in a hybridization mix (50% formamide/15% dextransulfate/2X SSC). The suspension was hybridized for 2 days at 37° C onto metaphase chromosome spreads. On completion of hybridization, the slides were washed, and the chromosomes were counterstained with 4´, 6´-diamidino-2 phenylindole (DAPI) to allow for their identification. Image analysis was performed in the following way: ten individual metaphases were captured for each case with a cooled-charged couple-device camera attached to a Nikon Microphot-SA microscope and processed by Quantitative Imaging Processing System (QUIPS, Applied Imaging, Santa Clara, CA). The chromosomes were identified by 4´, 6´-diamidino-2 phenylindole (DAPI) banding analysis, segmented, the local background substracted, and the median axis identified. Red, green and blue fluorescence was analyzed for all metaphase spreads, normalized to a standard length, and statistically combined to show the red: green signal ratio and 95% confidence intervals for the entire chromosome. Copy number changes were detected on the basis of the variance of the red-green ratio profile from the standard of 1. Ratio values of 1.2 and 2.0 were defined as thresholds for gains and high-level amplifications respectively. Losses were identified as ratios of 0.8 or less.

Statistical analysis

The relative risk of local relapse associated with the individual chromosomal alterations was estimated by comparison of the case with that of the matched controls, by conditional logistic regression methods for individually matched case-control studies[20]. Differences in high-level amplifications were calculated with help of the chi-square and McNemar tests. Relative-risk estimates, two-sided p-values, and 95% confidence intervals (CI) were calculated using SPSS 12.0.1. Due to the small number of cases, multivariable analysis was not performed.

RESULTS

Characteristics of study population

Six chemoradiation resistant tumors could not be used for CGH analysis because of insufficient DNA quantity (n= 4) and poor quality of paraffin DNA, manifest as poor quality of hybridization images (n= 2). As a result, the CGH data of 20 chemoradiation resistant and 20 chemoradiation sensitive tumors (matched-controls) were analyzed. Patients' characteristics are detailed in Table 1.

TABLE 1 Patient population of CGH analysis

Variable	chemoradiation	
	sensitive	resistant
Gender		
Male	15	16
Female	5	4
T- classification		
T3	5	1
T4	15	19
Tumorvolume		
Mean (range)	34 (11-86)	43 (10-102)
Median	28	38
N- classification		
N0-N1	8	9
N2-N3	12	11
TNM-stage		
Stage III	3	0
Stage IV	17	20
Site		
Oral cavity	4	6
Oropharynx	13	11
Hypopharynx	3	3
Infusion mode		
Unilateral	10	6
Bilateral	10	14

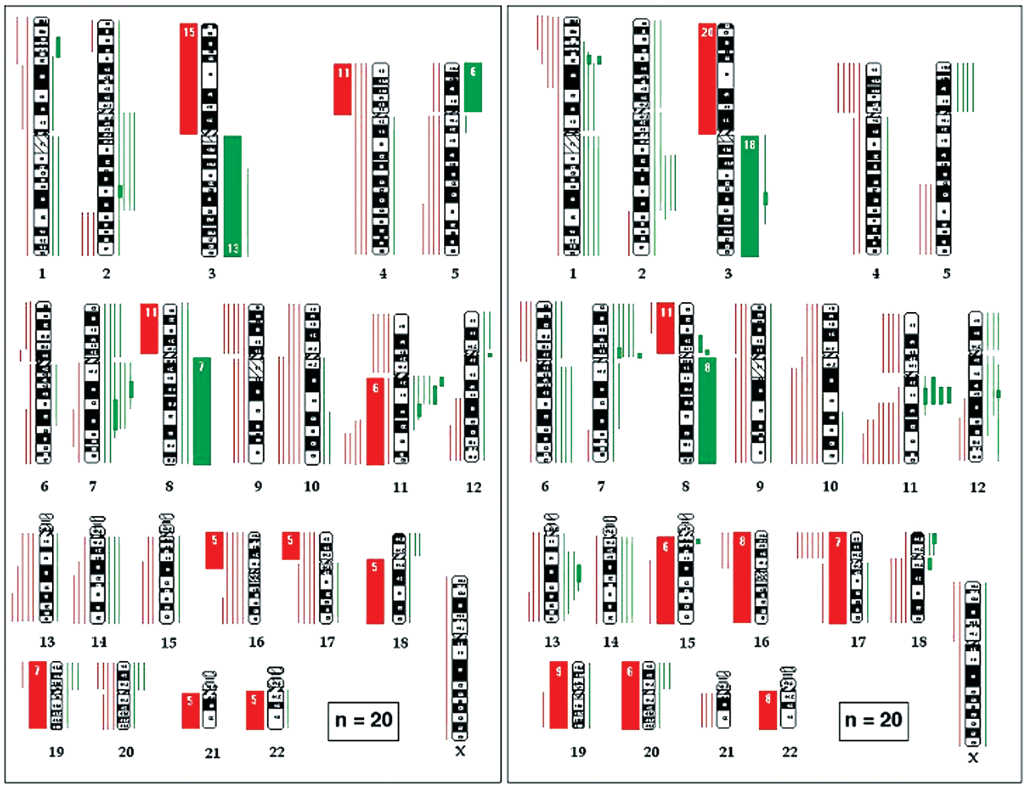
TABLE 2 High level amplifications in sensitive (N= 20) and resistant (N= 20) tumors

	Sensitive	Resistant
Number of patients with high level amplifications*	5	10
Number of high level amplifications**	8	16

* Chi-square: p= 0.01
** McNemar: p= 0.30

Comparison of chromosomal instability in chemoradiation sensitive and resistant cases

Chromosomal alterations were detected in all 40 tumors. Chemoradiation resistant and sensitive tumors did not differ significantly in the total number of detected chromosomal alterations (227 vs. 231), the number of detected chromosomal gains (82 vs. 71) or the number of detected chromosomal deletions (129 vs. 152). However, chemoradiation resistant cases were more often characterized by the presence of high-level amplifications as



sensitive resistant

FIGURE 1. Ideogram showing DNA copy number changes identified by CGH analysis of 20 chemoradiation-sensitive squamous cell carcinomas. Thin vertical lines on either side of the ideogram indicate losses (left) and gains (right) of the chromosomal region. Large thick lines with numbers represent the number of losses (left) and gains (right) corresponding the number within the thick line. Small thick lines without number represent chromosomal regions of the high-level amplification (right).

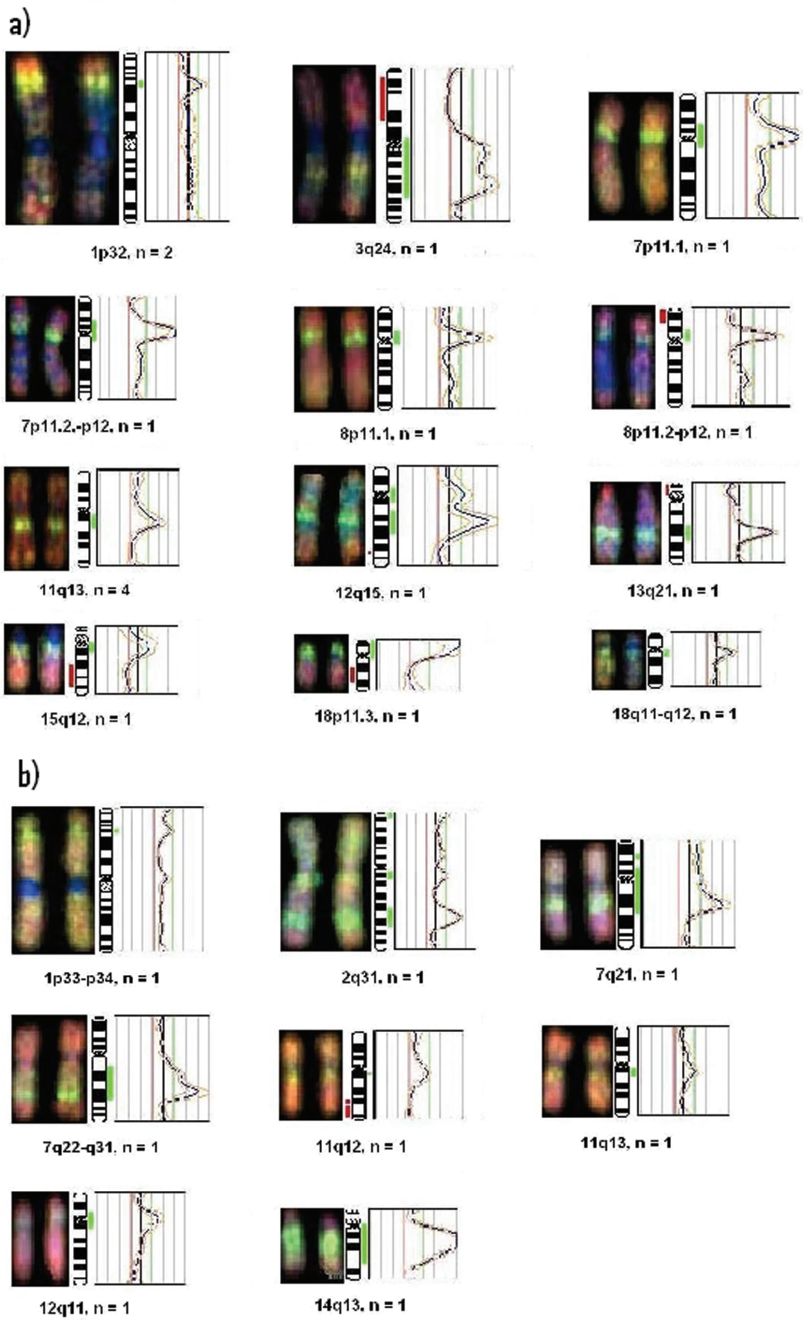


FIGURE 2. a) Partial karyotypes (left) and corresponding ratio profiles (right) illustrating high-level amplification of chromosomal regions in resistant tumors. Hybridized tumor DNA was visualized via FITC (green) and control DNA was visualized via Texas Red (red). The average green : red fluorescent ratio along the length of the chromosome is shown. b) Partial karyotypes (left) and corresponding ratio profiles (right) illustrating high-level amplification of chromosomal regions in sensitive tumors. Hybridized tumor DNA was visualized via FITC (green) and control DNA was visualized via Texas Red (red). The average green : red fluorescent ratio along the length of the chromosome is shown.

TABLE 3 Distribution of genetic abnormalities and their association with chemoradiation resistance in 20 case subjects and 20 chemoradiation sensitive matched control subjects with squamous cell carcinoma

Chromosomal abnormality	Cases No. (%)	Controls No. (%)	OR (95% CI)	p-value
3p11-pter loss				
present	20 (100)	15 (75)	1.00*	
not present	0 (0)	5 (25)	0.57 (0.11-1.03)	0.016
3q11-q13 gain				
present	19 (95)	13 (65)	1.00*	
not present	1 (5)	7 (35)	0.47 (0.09-0.85)	0.017
3q21-q26.1 gain				
present	19 (95)	14 (70)	1.00*	
not present	1 (5)	6 (30)	0.43 (0.03-0.84)	0.038
4p11-pter loss				
present	15 (75)	4 (20)	1.00*	
not present	5 (25)	16 (80)	0.55 (0.28-0.83)	<0.001
6q22-27 gain				
present	4 (20)	0 (0)	1.00*	
not present	16 (80)	20 (100)	0.56 (0.04-1.07)	0.036

* Reference category. OR: odds ratio. CI: confidence interval

compared their chemoradiation sensitive counterparts (10 vs. 5, p=0.01; Table 2). Also, the total number of detected high-level amplifications was higher in chemoradiation resistant cases (16 vs. 8, p=NS, Table 2).

Comparison of chromosomal profiles of chemoradiation resistant and chemoradiation sensitive cases

A comparison of individual chromosomal alterations detected in chemoradiation resistant and sensitive cases is shown in figure 1. Chromosomal aberrations differentiating chemoradiation resistance and chemoradiation sensitive cases included gains of 5q11-q12, 6q23-q27, 8p21-p23, 10q11-q22, 15q13-q26, 18q21-q23 and 22 and losses of 2p22-p25, 5p11-pter and 7q11-q22, (present in sensitive cases) and gains of 6p11-pter, 9 and Xq11-qter and loss of 18p11-pter (present in resistant cases). Statistical analysis of frequency distributions of individual chromosomal alterations revealed that gains of 3q11-q13 (p= 0.017), 3q21-q26.1 (p= 0.038), and 6q22-q27 (p= 0.036) and losses of 3p11-pter (p= 0.016) and 4p11-pter (p< 0.001) were significantly more common in chemoradiation resistant HNSCC (Table 3). In addition, further analysis revealed that chemoradiation resistant HNSCC (amplifications of 1p32, 3q24, 7p11.1, 7p11.2-12, 8p11.1, 8p11.1-12, 12q15, 13q21, 15q12, 18p11.3 and 18q11) and chemoradiation sensitive cases (1p33-34, 2q31, 7q21, 7q22-31, 11q12, 12q11 and 14q13) were characterized by a completely different profile of high-level amplifications (Table 2, Figure 2).

TABLE 4 p16 overexpression in sensitive (N= 20) and resistant (N= 20) tumors

	Sensitive	Resistant	Total
Number of patients without p16 overexpression	11	11	22
Number of patients with p16 overexpression	8	3	11
Not known	1	6	7
Total	20	20	40

DISCUSSION

The potential of human cancer cells to adapt to environmental selection pressures such as chemotherapy and radiotherapy is a major determinant of clinical treatment failure and associated survival reduction. In recent years, several molecular pathways have been identified that may mediate cellular responses to cytotoxic stress. In some instances, cancer cells may alter these pathways in their favor. Nonetheless, our understanding of resistance to cytotoxic agents is far from complete.

In the present study we report a genome-wide exploration of chemoradiation resistance. Our data suggest that chemoradiation sensitive and resistant HNSCCs do not differ in the overall number of chromosomal alterations present in the genome of tumor cells. In the literature, the relationship between treatment resistance and chromosomal damage is conflicting. Some previous studies have suggested that tumors resistant to chemotherapy or radiotherapy are characterized by a higher number of chromosomal alterations. For example, Akervall and colleagues[15] found a higher number of chromosomal alterations in cisplatin resistant HNSCC cell lines as compared to their sensitive counterparts. These studies suggest that development of chromosomal instability facilitates adaptation of tumor cells to cytotoxic agents. In contrast, a significant number of studies report a lower overall number of genetic alterations in chemotherapy resistant tumors suggesting that chromosomal instability makes tumor cells more vulnerable to cytotoxic agents[21]. Although it is difficult to compare our concomitant chemoradiation data to genetic data of patients treated on separate chemotherapy or radiation protocols, all cases were characterized by multiple chromosomal alterations, possibly influenced by their uniformly advanced tumorstage[22,23]. Therefore, we believe our data do not contribute to an increased understanding of the suspected link between chromosomal instability and cellular adaptability to cytotoxic stress.

In addition to a possible relationship between the overall number of genetic changes and chemotherapy or radiation therapy response, several studies have suggested that resistance to these treatments may be mediated by a higher number of gene amplifications in treatment resistant tumors. Rao et al.[19] found several high-level amplifications in germ cell tumors, which were restricted to cisplatin resistant tumors only. Wang and colleagues[14] reported that genetic amplification of thymidylate synthase defines a subgroup of colorectal cancers resistant to 5-fluorouracil. In agreement with these findings, we observed that high-level amplifications are more common in chemoradiation resistant tumors. This study demonstrated that not only the number of patients with high-level amplifications (10 vs. 5) was higher in the resistant-

group compared to the sensitive-group, but also the number of high-level amplifications (16 vs. 8; Table 2). The exact meaning of these findings in the context of chemoradiation resistance will depend on the identification of the genes driving selection for these high-level amplifications and their functional annotation.

In addition to the overall number of chromosomal alterations, our study identified several specific chromosomal alterations that may be associated with response to chemoradiation treatment including gains of 3q11-q13, 3q21-q26.1 and 6q22-q27 and losses of 3p11-pter and 4p11-pter that were significantly more common in chemoradiation-resistant tumors than in chemoradiation-sensitive tumors. The presence of squamous cell carcinoma-related oncogene (SCCRO) in the 3q26 region may be an explanation for the observed relationship between 3q21-26 overrepresentation and chemoradiation resistance[24,25]. SCCRO drives selection for 3q26 overrepresentation in squamous cell carcinomas and is a key activator of Hedgehog signaling which has been associated with chemoradiation resistance of squamous cell carcinomas[26]. In addition to 3q, loss of 3p has been linked to cytotoxic treatment resistance previously. Akervall and colleagues[15] reported loss of 3p associated with cisplatin-resistant HNSCC cell lines. In addition, Ogawa and colleagues[27] observed that loss of heterozygosity at 3p21 differentiated radiation-resistant from -sensitive laryngeal cancers and was inversely related to larynx preservation. The 3p21.3 region harbors various genes (e.g. FUS1, RASSF1A, 101F6, NPRL2)[28], that play a role in cell proliferation, cell cycle kinetics, signalling transduction, ion transportation and exchange, apoptosis and cell death. These genes, when deleted, may well modify chemoradiation sensitivity. In contrast to loss of 3p, no prior studies have directly associated gain of 6q or loss of 4p with cytotoxic treatment resistance of HNSCC. Although the 4p and 6q region are commonly altered in squamous cell carcinomas, no candidate genes have been described.

A number of limitations from this study should be addressed. Firstly, our results are based on a relatively small number of cases and matched controls. Based on this shortcoming and the significant number of matching criteria, it was not possible to match cases and controls perfectly. Although we did not find a statistically significant difference between the case and control group in individual matching criteria, the possibility of a significant difference in the overall profile or influence by other, not included factors remains an item of concern. For example, a possible difference in HPV-positivity between cases and controls could in principle account for the detected genetic differences[29]. This possibility is limited given the equal distribution of tonsillar carcinomas over the two groups. However, we assessed the frequency distribution of p16 immunopositivity, an established marker of HPV positivity. This analysis did not reveal evidence for HPV bias (Chi-square: p= 0.21, see Table 4). Therefore, we have no indication that the case and control group differ significantly in individual clinicopathological factors or their collective clinicopathological profile, but the possibility remains difficult to exclude entirely. In addition to issues of matching, the examination of pretreatment biopsies harbors a potential limitation. It is conceivable that genetic aberrations that cause chemoradiation resistance develop during treatment and a study comparing the genomic content of pre-treatment and post-treatment biopsies might address this issue

better. Given the study limitations, we believe external validation of our data is warranted. We are currently in the process of generating a tissue microarray of all patients included in the chemoradiation trial at the Netherlands Cancer Institute. This will allow for fluorescent in-situ-hybridization experiments and immunohistochemistry from a series of independent patients with known clinical outcome to validate the identified genetic factors as prognostic markers of chemoradiation outcome. Thereafter, identification of candidate genes and analysis of their function is needed to help explain the role of chromosomal alterations in chemoradiation resistance.

In summary, we conclude that different genetic abnormalities can be identified in chemoradiation resistant and chemoradiation sensitive tumors. Identification of amplified/ overexpressed genes at these sites may elucidate new genetic pathways of chemoradiation resistance in HNSCC.

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CHAPTER 4

Molecular markers predict outcome in squamous cell carcinoma of the head and neck after concomitant cisplatin-based chemoradiation

Guido B. van den Broek, Maarten Wildeman, Coen R.N. Rasch, Nicola Armstrong, Ed Schuuring, Adrian C. Begg, Leendert H.J. Looijenga, Rik Scheper, Jacqueline van der Wal, Lorian Menkema, Alfons J.M. Balm, Marie-Louise F. van Velthuysen, Michiel W.M. van den Brekel

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ABSTRACT

BACKGROUND. Not all patients with squamous cell carcinomas of the head and neck (HNSCC) benefit from concurrent cisplatin-based chemoradiation, but reliable predictive markers for outcome after chemoradiation are scarce. We have therefore investigated several potential prognostic biomarkers for outcome (local control and overall survival) in a large group of patients.

METHODS. Out of one hundred and four biopsies taken from consecutive HNSCC patients treated with concurrent cisplatin-based chemoradiation, ninety one tumor biopsies were evaluated for protein expression on a tissue micro-array. Using immunohistochemistry, 18 biomarkers, involved in various cellular pathways, were investigated: TP53, murine double minute 2 (MDM2), TP73, BCL-2, cyclin D1, cortactin, P21, P16, P27, retinoblastoma (RB), ki-67, epidermal growth hormone receptor, cyclooxygenase-2, hypoxic inducible factor 1alpha (HIF-1α), carbonic anhydrase IX, xeroderma pigmentosum protein group A (XPA), P-glycoprotein (MDR1) and multidrug resistance-associated protein 2 (MRP2). Univariable and multivariable proportional hazard analyses were performed to investigate associations between each individual marker and outcome. In addition, the global test was used to test all variables simultaneously and selected combinations of markers for an overall association with local control.

RESULTS. Univariable proportional hazard models showed statistically significant increased relative risks of RB, P16 and MRP2 for local control and MDR1 and HIF-1α for overall survival. MRP2, MDR1 and P16 levels were positively associated with outcome whereas RB and HIF-1α had a negative relationship. Using Goeman's global testing no combination of markers was identified that was associated with local control. Grouping the markers according to their function revealed an association between a combination of three markers (P16, P21, and P27) and outcome (ρ=0.05) was found. After the multivariable analysis MRP2 and RB remained significant independent predictive markers for local control.

CONCLUSIONS. This study describes the possible prognostic value of 18 biomarkers for the outcome in patients uniformly treated with concurrent chemoradiation. MRP2 and RB were found to be associated with outcome in patients treated with concurrent chemoradiation.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common cancer in men[1]. Over 70% of head and neck cancer patients present with advanced stage III and IV disease. Concomitant chemoradiation (CCRT) leads to improved local control and overall survival in advanced head and neck cancer compared with conventional radiotherapy[2-7], making this modality the most suitable curative treatment option in these patients currently. However, CCRT is not effective in all patients, and when unsuccessful, patients suffer the potential side effects and toxicities of chemotherapy (e.g. swallowing problems, hearing loss) and radiation therapy (e.g. mucositis, late toxicity). Therefore, identification of reliable outcome predictors in this setting is of clinical interest and especially important if alternatives such as surgery and postoperative radiotherapy or cetuximab with radiotherapy are possible.

Clinical variables have been intensively studied for prognostic accuracy. TNM classification is universally used as a staging system. However, in patients with advanced head and neck cancer it has been demonstrated that T and N stage do not have sufficient predictive value[8,9]. Tumor volume has been proven to be the most predictive factor for local control[8-12]. For overall survival, tumor volume, co-morbidity, lowest involved neck level and pre-treatment weight loss have been found to be prognostic. Although tumor volume has strong prognostic value, we have demonstrated that tumors with the same volume can have different outcomes[13]. These and other findings[14] suggest that other biological factors are important in determining tumor response and knowledge of these and other factors could be helpful in clinical decision making. These can be hypoxia, repopulation rate and intrinsic sensitivities to radiation and cisplatin (e.g. repair pathways and drug pumps), amongst others.

Recent studies have described many biological markers correlating with outcome[13,15-27]. However, only a few have been tested for predictive accuracy after chemoradiation treatment. Such markers include those associated with cell cycle control (cyclin D1, retinoblastoma gene product (RB), P16, P21 and P27), apoptosis (TP53, MDM2, TP73 and BCL-2), growth regulation (EGFR), cyclooxygenase-2 (COX-2) and ki-67), focal adhesion signaling (cortactin), hypoxia (CA9, HIF-1α) and sensitivity to chemotherapy (XPA, MRP2 and MDR1).

Tissue micro-array (TMA) technology in combination with immunohistochemistry has been demonstrated to be an appropriate tool to analyze the prognostic value of genetic abnormalities in a large number of tumor samples simultaneously[28-31]. In this study we investigated the role of several biomarkers in predicting local control (LC) and overall survival (OS) in patients with squamous cell carcinoma after treatment with chemoradiation. These markers were chosen as the most promising individual markers in previous studies. We also conducted a review of the published literature in order to assess and compare the expression and prognostic value of biomarkers in patients with advanced head and neck cancer treated with radiotherapy, chemotherapy or a combination of both modalities. Our data suggest that only a few molecular markers known from current head and neck literature might play a role in chemoradiation resistance.

PATIENTS AND METHODS

Patients and tissue samples

From 1997 until 2000, 104 consecutive patients with advanced squamous cell carcinoma of the oral cavity, oropharynx, supraglottic larynx and hypopharynx were treated in two chemoradiation trials (RADPLAT). Paraffin embedded biopsies of the primary tumor were available for immunohistochemical analysis in 95 of these patients.

The intra-arterial chemoradiation treatment has been described earlier[32,33]. Briefly, treatment consisted of four consecutive weekly selective intra-arterial infusions of cisplatin (150 mg/m^2) simultaneous with intravenous sodium thiosulfate rescue combined with radiotherapy (2 Gy per day, 5/week x 7 to a total dose of 70 Gy) according to the RADPLAT protocol[34]. Before the start of treatment, all patients signed an informed consent form approved by our institutional protocol review committee. Treatment response was evaluated six to eight weeks after completion of radiotherapy by magnetic resonance imaging, followed by examination under general anesthesia. Thereafter, patients were subjected to regular outpatient follow-up and an annual chest X-ray. To decrease the chance of a late recurrence, all living patients had a minimum follow-up period of two years. Patient characteristics are detailed in Table 1.

Methods

The markers have been chosen to reflect different biological processes that might be involved in the response to concurrent chemoradiation. We performed a literature search to compare expression and prognostic value of several molecular markers. The following search terms were used: squamous cell carcinoma, head and neck neoplasm, immunohistochemistry, and outcome for each molecular marker. Studies describing less than 25 cases, any surgical treatment, esophageal or nasopharyngeal cancer were excluded. Based on these criteria, we selected the following markers: BCL-2[17,26,35-40], CA9[18-20,41], COX-2[42], cyclin D1[17,23,35], EGFR[43,44], HIF-1α[20,45], ki-67[17,21-23,35,38,39,42,46,47], MDM2[48,49], MRP[24], P16[23], P21[23,49-51], P27[23,51], TP53[16,17,21-25,35,36,38,39,41,42,45,46,48-53] and RB[23].

Immunohistochemistry

Tissue micro-arrays were constructed as described by Chen et al.[30]. Briefly, an H & E slide from the tumor embedded paraffin block was used to guide the sampling of morphologically representative regions of the tumor. To construct the tissue micro-array, three core tissue biopsy specimens (diameter: 0.6 mm) from the selected regions of the donor block were taken and precisely arrayed into a new recipient paraffin block. Each micro-array block contained a maximum of 168 punches and two paraffin blocks were produced. After the construction of the array block, 5 µm paraffin serial sections were cut with a microtome using an adhesive-coated tape sectioning system (Instramedics Hackensack, NJ) of which one H & E staining was performed to verify histology.

Staining with antibodies was performed using standard methodologies previously described[30,54,55]. In short, paraffin embedded, formalin fixed sections were deparraffinized

and antigen retrieval was performed (Table 2). After blocking endogenous peroxidases with 0.3% H_2O_2 the sections were stained for primary antibody diluted in 1% BSA-PBS. Secondary rabbit anti-mouse peroxidase or goat anti-rabbit peroxidase were precipitated using 3.3' diaminobenzidine tetrachloride as a substrate and slides were counterstained using routine hematoxyline.

Immunohistochemistry scoring

Tissue micro-arrays contained 3 cores from each tumor biopsy taken from every patient prior to therapy. Immunohistochemical stainings were scored by two independent observers (G.B.vd.B, M.W.). The scorings were randomly checked by the pathologist and in case of disagreement between both observers, after discussion with a pathologist (M.v.V.) the final score was determined upon general agreement. Staining of all antibodies was evaluated for both positivity percentage and intensity independently. The score for positivity was the percentage of positive cells averaged over three cores. Intensity scoring was performed using 4 categories (-; +; ++; +++).

Statistical methods

Reproducibility of the tissue micro-array scores was determined from the differences between the three cores and primarily expressed using the intraclass correlation coefficient (ICC). The ICC was calculated from the three cores and expresses the percentage of overall variance. Positivity scores, taken as continuous percentages, were used for the statistical analysis. In other words, no cut off values were used in scoring or to analyze expression. Time was calculated from start of treatment until local failure, end of follow-up or death.

Due to the large number of variables (N=18) compared to the number of events (N=16), methods developed for cDNA micro-arrays were used. The global test of Goeman[56] was used to test all 18 variables simultaneously or a combination of some related variables for an overall association with local control. Combinations of related variables were chosen according to known specific pathways: cell cycle control (cyclin D1, cortactin, RB) and (P27, P16, P21); apoptosis (TP53, TP73, MDM2, P21); hypoxia (HIF-1α, CA9) and chemotherapy sensitivity (XPA, MRP2, MDR1). 10000 permutations were used to calculate the *p*-value. Reproducibility, univariable and multivariable analyses of outcome and additional analyses were performed using the statistical package S+ (version 6.2 for Windows). Outcome curves were calculated using the Kaplan-Meier method[57]. To visualize the significant outcome differences using Kaplan-Meier curves, the continuous positivity scores were converted to categorical scores (low/high). Cox proportional hazard analyses, both univariable and multivariable were performed using the continuous positivity scores[58]. Clinical factors like T-classification, N-classification and site were included in the multivariable analysis. The statistical package R (version 2.2.1 and 2.4.1) was used to perform the global test.

RESULTS

In four patients, tumor tissue appeared to be of insufficient quality to be used in the analysis (defined as more then 20 missing values among the 36 final intensity and final percentage scores). These subjects were excluded from all analyses and the other 91 tumors were deemed to be of sufficient quality to be included in the analysis. Median follow-up for overall survival was 18 months (range 1-58 months). The median age of the patients was 54 years (mean = 56, range 29-78). During follow-up, sixteen patients had a local recurrence and fifty-three patients died. Two-year local control and overall survival rates were 78% and 48%, respectively. The median age of the patients was 54 years (mean = 56, range 29-78). Other patient and tumor characteristics are detailed in Table 1.

Immunohistochemical scoring by intensity had poor reproducibility in general (ICC < 0.5). The reproducibility of cores scored with the positivity-method was moderate (ICC 0.5- 0.8) to good (ICC >0.8). Data for expression of the markers are shown in Table 3. Several markers were expressed in many tumors (e.g. EGFR, HIF-1α, cortactin and RB), whereas others demonstrated immunoreactivity in only a limited number of tumors (e.g. BCL-2, MRP2 and P16).

Since the reproducibility of markers scored by the positivity-method was much better than the intensity-scoring method, we used only the positivity scores for further analyses. From the univariable analysis, three markers were found to have a significant predictive value for local control: RB (p= 0.036), P16 (p= 0.008) and MRP2 (p= 0.007; Table 4). MRP2 and P16 expression had a positive association with local control (Figure 1 and 2), whereas RB expression had a negative relationship with local control. According to the global test of Goeman, a combination of all markers did not show an association with local control. Several

TABLE 1 Patient population

variable	number	percentage (%)
Gender		
Male	70	77
Female	21	23
T- classification		
T3	19	21
T4	72	79
N- classification		
N0-N1	30	33
N2-N3	61	67
TNM-stage		
Stage III	7	8
Stage IV	84	92
Site		
Oral cavity	15	16
Oropharynx	57	62
Hypopharynx	19	22

TABLE 2 Details on primary antibodies used for immunohistochemical stainings

antigen	antibody	species	source	localization of staining
Cyclin D1	SP4	Rabbit	NeoMarkers	Nuclear
Cortactin	30/cortactin	Mouse	BD Transduction Laboratories	Membranous/cytoplasmic
P21	P21WAF-1/EA10	Mouse	Oncogene	Nuclear
P27	1B4	Mouse	NovoCastra	Nuclear
COX2	33	Mouse	BD Transduction Laboratories	cytoplasmic
HIF-1α	54/HIF-1α	Mouse	BD Transduction Laboratories	nuclear / membranous
CA9	M75	Mouse	Bayer	membranous / cytoplasmic
BCL-2	Bcl-2α AB-3	Mouse	Neomarkers	cytoplasmic
RB	G3-245	Mouse	Pharmingen	nuclear
EGFR	MS-378	Mouse	Neomarkers	membranous
P16	MS-887	Mouse	Neomarkers	nuclear
TP53	DO-7	Mouse	Dako	nuclear
ki-67	MIB-1	Mouse	Immunotech	nuclear
XPA	XPA AB1	Mouse	Neomarkers	nuclear
MRP2	M2III5	Mouse	Rik Scheper	membranous
Pgp	JSB1	Mouse	Rik Scheper	membranous
MDM2	Clone sample 14	Mouse	Neomarkers	nuclear
TP73	P73 (H-79)	Rabbit	Santa Cruz	nuclear

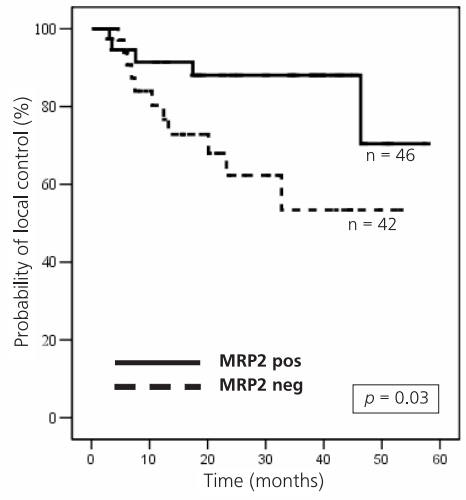

FIGURE 1. Kaplan-Meier curves of 88 patients stratified for MRP2. Cut off value: 0%, high expression (——) and low expression (·····). Log-rank test: p = 0.03.

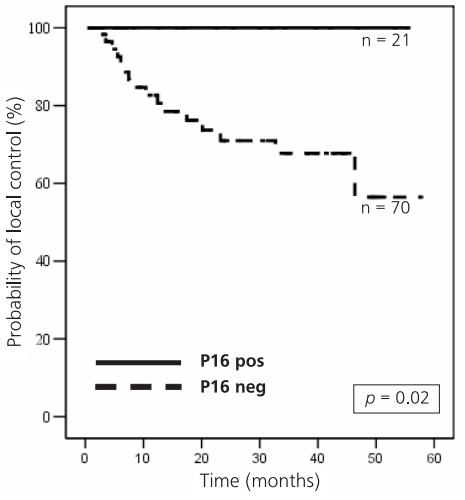

FIGURE 2. Kaplan-Meier curves of 91 patients stratified for P16. Cut off value: 25%, high expression (——) and low expression (·····). Log-rank test: p = 0.02.

TABLE 3 Marker characteristics (positivity percentage)

variable	number	mean (%)	median (%)
HIF-1α	86	78	88
P21	88	64	74
COX2	83	55	88
cortactin	90	74	88
cyclin D1	90	33	31
P27	90	64	68
MDM2	91	66	68
MRP2	91	20	3
XPA	89	66	81
TP73	88	59	68
P16	91	20	0
TP53	88	57	88
ki-67	91	57	68
RB	90	73	88
EGFR	88	82	88
CA9	87	41	45
bcl-2	90	13	0
P-glycoprotein	90	23	0

related groups of genes were then tested for prognostic value. The combination of P27, P21 and P16 was found to be significantly related to local control (permutations *p*-value= 0.03). MRP2, RB, P16, P21, P27 and clinical factors (T-classification, N-classification and site) were included in the multivariable analysis for local control. Because patients with a local recurrence had only stage IV disease, TNM stage was not included. After the multivariable analysis, MRP2 and RB remained as independent significant variables and P16 was borderline significant (p= 0.06) for local control (Table 5).

When overall survival was considered as the outcome, two markers as well as gender (p= 0.03; Table 6) were found to be significantly prognostic in univariable analysis: HIF-1α (p= 0.03), MDR1 (p= 0.04). MDR1 expression had a positive association with overall survival, whereas HIF-1α expression had a negative relationship with overall survival. HIF-1α, MDR1 and clinical variables were included in a multivariable analysis for overall survival. This analysis demonstrated that HIF-1α remained borderline significant as a prognostic marker for overall survival (p= 0.053, Table 7).

TABLE 4 Univariable analysis for local control

variable	p- value	Hazard Ratio	95% Hazard Ratio Confidence Limits
MRP2	0.007	0.32	0.15 - 0.70
P16	0.008	0.27	0.10 - 0.77
RB	0.04	2.10	1.08 - 4.09
P27	0.09	1.60	0.99 - 2.60
HIF-1α	0.12	1.56	0.89 - 2.74
CA9	0.23	0.73	0.43 - 1.23

TABLE 5 Multivariable analysis for local control*

variable	p- value	Hazard Ratio	95% Hazard Ratio Confidence Limits
MRP2	0.005	0.28	0.11 - 0.68
RB	0.04	1.77	1.03 - 3.03
P16	0.06	0.26	0.06 - 1.08

* T-classification, N-classification, site, P21 and P27 were included in the model as well, but were eliminated in a backward manner.

TABLE 6 Univariable analysis for overall survival

variable	p- value	Hazard Ratio	95% Hazard Ratio Confidence Limits
HIF-1α	0.03	1.38	1.03 - 1.83
gender (male vs. female)	0.03	0.48	0.25 - 0.92
MDR1	0.04	0.76	0.58 - 0.98
MRP2	0.07	0.76	0.54 - 1.08
XPA	0.07	1.29	0.95 - 1.76

TABLE 7 Multivariable analysis for overall survival

variable	p- value	Hazard Ratio	95% Hazard Ratio Confidence Limits
HIF-1α	0.053	1.33	0.99 - 1.77
MDR1	0.11	0.80	0.61 - 1.05
N-classification (N0-1 vs. N2-3)	0.03	1.39	1.04 - 1.85
T-classification (T3 vs. T4)	0.09	1.30	0.96 - 1.77
gender	0.14	0.79	0.61 - 1.03
site:			
oral cavity		1.00	
oropharynx	0.27	0.82	0.57 - 1.17
hypopharynx	0.41	0.86	0.61 - 1.23

TABLE 8 Literature review: prognostic value of markers for outcome

marker	author (et al.)	N	site	treatment	cut off (%)	expression (%)	LC	u/m	OS	u/m
bcl2	Trask [26]	47	larynx	CT	>50	15	no*		no	
	Homma [39]	59	larynx	CCRT	>30	12	no		yes	m
	Gallo [37]	85	all	RT	>30	24	yes	m	yes	m
	Homma [38]	111	all	CCRT	>30	13	yes	m	no	
	Nix [40] **	124	larynx	RT	>5	32	yes	m	nm	
	Fouret [36]	139	all	CT	>5	18	yes*	m	nm	
	Ataman [35]	309	all	RT	>5	13	yes	m	nm	
	Buffa [17]	402	all	RT	>5	13	yes	m	yes	m
CA9	Kaanders [19]	38	all	RT	nm	nm	no		no	
	Schutter [18]	67	all	RT	>17	50	no		no	
	Koukourakis [20]	75	all	CCRT	strong staining	27	yes	u	yes	u
	Koukourakis [87]	198	all	RT	>10	58	yes	m	yes	m
COX-2	Cho [42]	123	larynx	RT	int(3) #	46	no		yes	m
Cyclin D1	Rodriguez [23]	122	all	CCRT	>5	64	no		no	
	Ataman [35]	309	all	RT	continuous	100	no		nm	
	Buffa [17]	402	all	RT	continuous	80	no		yes	m
EGFR	Demiral [43]	31	larynx	RT	>5	16	yes	u	nm	
	Gupta [44]	38	oropharynx	CCRT	int(4) #	79	no		no	
HIF-1α	Koukourakis [20]	75	all	CCRT	>36	52	no		no	
	Aebersold [15]	98	oropharynx	RT	>0	94	yes	m	yes	m
ki-67	Valente [47]	31	oral cavity	RT	>50	nm	no		nm	
	Raybaud [22]	56	all	RT	>20	32	yes	m	nm	
	Homma [39]	59	larynx	CCRT	>50	49	no		no	
	Lavertu [21]	105	all	CCRT	>0	24	no		yes	m
	Homma [38]	111	all	CCRT	>40	67	no		no	
	Rodriguez [23]	122	all	CCRT	>20	53	no		no	
	Cho [42]	123	larynx	RT	>10	28	no		yes	m
	Couture [46]	304	all	RT	>20	59	no		no	
	Ataman [35]	309	all	RT	>20	53	no		nm	
	Buffa [17]	402	all	RT	<20; 20-40; >40	46; 32; 22	no		no	
MDM2	Osman [49]	71	all	CCRT	>20	74	no		yes	m
	Friesland [48]	70	tonsil	RT	int(4) #	nm	no		no	
MRP	Shiga [24]	68	all	CT + RT	>5	43	no		no	
p16	Rodriguez [23]	122	all	CCRT	>50	68	no		no	
p21	Jeannon [50]	60	larynx	RT	>50	58	nm		yes	u
	Korkmaz [51]	68	larynx	RT	>10	60	no		no	
	Osman [49]	71	all	CCRT	>20	54	no		no	
	Rodriguez [23]	122	all	CCRT	>10	34	no		no	
p27	Korkmaz [51]	68	larynx	RT	>10	37	yes	m	no	
	Rodriguez [23]	122	all	CCRT	>25	45	no		no	

marker	author (et al.)	N	site	treatment	cut off (%)	expression (%)	LC	u/m	OS	u/m
TP53	Raybaud [22]	56	all	RT	>10	41	yes	m	nm	
	Homma [39]	59	larynx	CCRT	>10	59	no		no	
	Jeannon [50]	60	larynx	RT	>25	48	nm		no	
	Narayana [52] **	67	larynx	RT	>10	46	yes	u	nm	
	Korkmaz [51]	68	larynx	RT	>10	61	no		no	
	Shiga [24]	68	all	CT + RT	>5	37	no		yes	m
	Osman [49]	71	all	CCRT	>20	49	no		yes	m
	Friesland [48]	75	tonsil	RT	>10	55	no		no	
	Bradford [16]	94	larynx	CT + RT	>0	57	no		no	
	Koukourakis [41]	95	all	(C)CRT	>20	39	no		no	
	Aebersold [45]	100	oropharynx	RT +/- CT	>10	67	no		no	
	Narayana [53]	102	larynx	RT	>10	37	yes	m	no	
	Lavertu [21]	105	all	CCRT	>2	55	no		no	
	Temam [25]	105	all	CT	>5	61	no*		nm	
	Homma [38]	111	all	CCRT	>10	55	no		no	
	Rodriguez [23]	122	all	CCRT	>10	55	no		no	
	Cho [42]	123	larynx	RT	>20	36	no		no	
	Fouret [36]	139	all	CT	>5	59	no*		nm	
	Couture [46]	304	all	RT	>10	44	yes	m	no	
	Ataman [35]	309	all	RT	<5; 5-75; >75	50; 25; 25	no		nm	
	Buffa [17]	402	all	RT	int(3) #	42	no		yes ‡	m
RB	Rodriguez(23)	122	all	CCRT	>10	81	no		no	

LC = local control; OS = overall survival; nm = not mentioned; u = univariable analysis; m = multivariable analysis;
* LC defined as >50% tumor response; ** case-control study; ‡ intermediate group; # intensity was scored

DISCUSSION

Several biomarkers have been described to be prognostic for outcome in squamous cell carcinoma of the head and neck[17,35,36,59-62], although most reported studies included HNSCC carcinomas that were treated surgically and the findings in many of these studies are contradictory[63]. In HNSCC cancer patients, chemoradiation is increasingly being used to treat locally advanced tumors. Therefore in this study, the prognostic value of 18 of the more promising biomarkers was investigated in tumors from HNSCC patients treated with concurrent chemoradiation. The markers were chosen because of their known role in chemosensitivity or radiosensitivity. Genes included those for cell cycle control, apoptosis, hypoxia, DNA repair, drug transport and growth signaling, all of which have been associated with drug or radiation response. Since there are a multitude of genes on these different pathways and processes, we chose representatives that had shown their value as predictors in previous studies, but had never been studied as a group, nor in patients treated with one of the emerging current treatment standards of concurrent high dose cisplatin and radiation.

A multivariable analysis showed that two biomarkers (RB and MRP2) were significantly and independently predictive for local control and one biomarker (HIF-1α) had prognostic value for overall survival. We also found that a combination of P16, P21 and P27 was significantly associated with local control, although P16 alone showed the strongest association.

The present study demonstrates the possible association between two markers (MRP2 and RB) and response to concurrent chemoradiation. MRP2 is a member of the ABC transporter family[64,65] which is able to export cisplatin out of the cell[66-68]. It would therefore be expected that tumors showing overexpression of MRP2 might be clinically chemoradiation resistant. However, we found increased expression of MRP2 in the sensitive tumors. Correlations between MRP2 expression and resistance to cisplatin have been demonstrated in *in vitro* studies[68,69]. In addition, downregulation of MRP2 using siRNA resulted in increased sensitivity to cisplatin[70]. Guminski et al.[71] examined MRP2 expression in normal cells and a series of ovarian carcinomas treated with platinum-based chemotherapy and demonstrated that MRP2 mediated efflux was a determinant of cisplatin sensitivity. However, the same group found higher MRP2 expression in platinum-sensitive carcinomas (55%) than in resistant cases (23%), consistent with our study and counter-intuitive.

Since tumor volume is a strong predictor of outcome[8,9] in advanced head and neck squamous cell carcinomas, we looked for a possible association between MRP2 levels and volume. However, mean tumor volumes were 43 and 37 cm^3 for the MRP2 positive and MRP2 negative cases, respectively, which are not significantly different. Another reason for the association of MRP2 expression and sensitivity to chemoradiation treatment could be the granular cytoplasmatic staining in MRP2 positive tumor cells. It is possible that cells not showing membrane localization do not have an efficient efflux mechanism due to incorrect protein localization. Another possible explanation is that MRP2 is known to pump reduced glutathione and conjugates out of the cell[72]. Tumors overexpressing MRP2 may therefore have lower glutathione levels, and since glutathione is the major radical scavenger protecting cells from oxidative DNA damage of the sort produced by ionising radiation, this could render them more sensitive to radiation[73]. This requires testing in cell culture models. Either way, our study indicates that expression of MRP2 has a role in, or is a marker of, sensitivity to cisplatin-based treatment in head and neck carcinomas.

The role of RB in predicting outcome in head and neck cancer has been investigated mostly in patients treated with surgery[74,75]. To our knowledge, only one study has been performed in chemoradiation patients and showed no firm association with treatment response[23]. Here we observed an inverse association with local control. Although this association was not as strong as for MRP2, patients with high RB expression had an increased probability of having a recurrence. A predictive value of P16 has been found in surgically treated patients[76-78], but we describe here an association between P16 and outcome in chemoradiation-treated patients. In our study, P16 was borderline significant in a multivariable analysis, independent of MRP2 expression and other clinico-pathological factors. Patients with high P16 expression had an increased probability of local control compared to patients with low P16 expression. P16 is a negative regulator of the cell cycle by acting as an inhibitor of cyclin-dependent

kinase 4 and 6-cyclin D complexes[79,80]. It has been hypothesized that cisplatin affects nuclear transport and stabilization of P16, which might partly explain the association with local control[81]. It is also an indirect marker of HPV, being expressed in HPV induced cancers but not in most other SCC, and HPV expressing SCC tumors have been found to be more sensitive to treatment[82,83].

HIF-1α was the only independent marker showing a strong trend related to overall survival in the multivariable analysis (borderline significance, p= 0.053). HIF-1α positive patients had worse survival than HIF-1α negative patients. This is in agreement with earlier published data in HNSCC. Aebersold et al.[15] found a significantly higher overall survival in oropharyngeal cancer patients showing increased HIF-1α expression. HIF-1α is induced by hypoxia, subsequently regulating erythropoiesis, glycolysis and angiogenesis that may promote survival, invasion and metastasis[84-86]. The stimulus for angiogenesis which promotes distant metastasis might explain why HIF-1α is associated with overall survival and not with local control.

The level of expression is potentially an important parameter for prognosis. Unfortunately, a search of the literature revealed a large variation in cut off values used for expression for most markers (summary in Table 8): BCL-2 (13-32%), CA9 (27-58%), COX-2 (46%), ki-67 (24-67%), P21 (34-60%), P27 (37-45%) and TP53 (36-67%). This makes it difficult to compare studies. Ideally a cut-off value should separate resistant cases from sensitive cases, but the ideal cut-off value is usually not known. Most investigators chose cut-off values to separate the study into comparable sized groups. However, because resistant cases are often a small portion of the study group, choosing cut-off values that differentiates a small subpopulation from a larger group might also be acceptable. Furthermore, it might well be that a small subpopulation of tumor cells determines resistance to a therapy. To partially circumvent these problems, we used a continuous variable instead of a cut off value for the analysis of expression of markers.

Despite variations in cut-off values, less than 40% of published studies reported an association between a predictive molecular marker and outcome after chemoradiation (Table 8). BCL-2 has been reported to be predictive for local control in more than half of all published studies. In four studies[17,35,36,40] the same cut off value (>5%) was used and a significant association between BCL-2 positivity and local control was observed. Using a cut off value of 30%, a significant association was also seen in two other studies[37,38]. The different cut-off values were not related to the different antibodies used. In our current study, if the expression of BCL-2 was categorized as positive or negative, with 5% taken as the cut-off value, a difference in local control rates was found (78% and 61%), although not significant (p = 0.39). Two other studies[26,38] also did not find an association with local control. Due to different reported expression and cut-off values, the value of BCL-2 as a tool for clinical decision making therefore remains weak, and the lack of predictive value found in our study cannot therefore be regarded as surprising.

As there is no single biomarker that appears to have repeatedly strong predictive value, several investigators have tried to find a combination of markers with a stronger predictive value. Gallo et al.[37] found a worse outcome in patients with mutated TP53 and positive BCL-

2 expression in head and neck squamous cell carcinomas. De Schutter et al.[18] demonstrated an association between local control and a combination of positive expression of CA9 and glucose transporter-I. We performed the global test of Goeman on selected groups of markers. One combination was found to be significantly associated with local control: P16, P21 and P27. However, after correction for multiple testing this association became weaker. A grouping of several biomarkers did not result in a better correlation with outcome compared to some biomarkers separately. It seems likely that multiple factors like DNA repair, apoptosis, cell cycle control, hypoxia and transmembrane drug pumps can contribute to chemoradiation-resistance, and the relative importance of these factors is likely to vary in different tumors, which makes identification of one strong prognostic molecular marker difficult. Prospective analysis of candidate markers and further investigation of their function is needed to help explain the role of chemoradiation resistance in head and neck squamous cell carcinoma.

In conclusion, this is the first study describing the possible prognostic value of 18 biomarkers for outcome in patients uniformly treated with concurrent chemoradiation. Although the exact role of MRP2 and P16 in the chemoradiation response in HNSCC has to be elucidated, both biomarkers were associated with clinical outcome. These markers need further validation in a similar patient group. In addition, it is unlikely that these are the best or the only possible markers for predicting outcome in this patient group, and it can be anticipated that present genome-wide screens (comparative genome hybridization, expression microarrays, epigenetics, etc) will lead to more and robust clinically useful markers in the future.

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CHAPTER 5

Response measurement after intra-arterial chemoradiation in advanced head and neck carcinoma: MRI and evaluation under general anesthesia?

Guido B. van den Broek, Coen R.N. Rasch, Frank A. Pameijer, Ellen Peter, Michiel W.M. van den Brekel, Alfons J.M. Balm

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ABSTRACT

BACKGROUND. To evaluate in a prospective trial, the diagnostic accuracy and predictive value of magnetic resonance imaging (MRI) and evaluation under general anesthesia (EGA) 6-8 weeks after chemoradiation on determining local control.

METHODS. Eighty-two consecutive patients with advanced stage squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx and supraglottic larynx were treated with selective-targeted chemoradiation (acronym: "RADPLAT"). All patients that completed treatment and survived had a minimum follow-up period of 3 years. MRI and EGA were performed 6 to 8 weeks after treatment. Posttreatment MRI findings were compared with pretreatment MRI findings and graded for risk of local recurrence/residual disease on a 4-point scale. The diagnosis of treatment failure was based on tissue biopsies, which were obtained during EGA or later during follow-up. The predictive value of MRI was analyzed using Cox proportional hazards model.

RESULTS. Only one patient with MRI grade 0 or 1 findings (discrete mass <10mm; n = 62) had residual disease 6-8 weeks after treatment, which was detected during EGA. In five patients with MRI findings of grade 2a and 2b (mass >10mm; n = 20) residual disease was detected. After 2 years, 23 patients had a local failure (28%). Twelve local failures were found among 62 patients with MRI findings of grade 0 and 1. Post-treatment MRI emerged as an independent predictive factor (HR 3.0; p = 0.014) for local control.

CONCLUSIONS. Posttreatment MRI provides predictive information on local control in addition to pretreatment predictors. In patients with focal masses < 10mm, the combination of response evaluation under general anesthesia and posttreatment MRI 6-8 weeks after chemo-irradiation, hardly provides more information on the local control than posttreatment MRI alone.

INTRODUCTION

A combination of concurrent chemotherapy and radiotherapy has become the treatment of choice in patients with advanced head and neck squamous cell carcinoma (HNSCC) with significantly improved locoregional control and overall survival compared to radiotherapy alone[1-7]. In patients with advanced stage III / IV disease this combined treatment is increasingly applied with curative intent. On average one third of these patients will develop a recurrent primary tumor[4,8]. In selected functionally irresectable cases early discovery of persistent disease or early recurrence may allow salvage surgery, which therefore requires accurate assessment of response. Reliable assessment of response is often difficult, due to the location of the primary tumor as well as to treatment induced changes such as non-healing mucosal defects, edema, fibrosis and complaints caused by these changes. Particularly in chemoradiation patients with substantial tumor volumes, these changes can be quite significant.

Posttreatment magnetic resonance imaging (MRI) and computer tomography (CT) have already proved to be valuable tools for detection of residual disease[9,10]. Optimal judgment is obtained if posttreatment images can be compared with pretreatment radiology[11,12]. The combination of imaging and examination under general anesthesia (EGA) including a biopsy seems even more appropriate.

In this article, we present the data on posttreatment evaluation of patients with advanced, inoperable squamous cell carcinoma participating in phase II and III intra-arterial chemoradiation trials (RADPLAT). Diagnostic accuracy and predictive value of MRI and the additional value of EGA performed 6-8 weeks after chemoradiation will be presented.

chapter 5

PATIENTS AND METHODS

Between April 1997 and May 2001, 105 consecutive patients with newly diagnosed inoperable T3-T4 squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx and supraglottic larynx were enrolled for targeted chemoradiation (RADPLAT). Details on this treatment modality have been described earlier[8,13,14]. Briefly, treatment consisted of four consecutive weekly selective intra-arterial infusions of cisplatin (150 mg/m^2) followed by intravenous sodium thiosulfate rescue combined with simultaneous radiotherapy according to the RADPLAT protocol[15] (2 Gy per day, 5/week x 7 to a total dose of 70 Gy). Before the start of treatment, all patients signed an informed consent form approved by our institutional protocol review committee.

To response to therapy and to allow for acute side effects to subside, treatment results were radiologically evaluated by MRI 6-8 weeks after completion of chemoradiation, immediately followed by EGA. MRI examinations were performed on a 1.5-Tesla scanner (Siemens Magnetom 63 SP4000; Siemens, Erlangen, Germany). The chosen section thickness was 4 mm or less, with an interslice gap of 1 mm or less. T1-weighted images were obtained before and after injection of intravenous gadolinium. Post-contrast images were acquired

TABLE 1 Grading of posttreatment MRI evaluation according to Ojiri et al.[16]

Grade 0	no detectable focal abnormalities, only expected post-chemoradiation changes
Grade 1	anatomic asymmetry or discrete mass £ 10 mm
Grade 2a	discrete mass > 10 mm
Grade 2b	<50% reduction of the largest dimension of the primary tumor between pre-chemoradiation and post-chemoradiation MRI studies

using fat-saturation. During EGA (MRI-guided) tissue biopsies were taken when the MRI was abnormal or when the area of interest showed any visual or palpable findings suspicious for residual disease. Only patients with biopsies showing squamous cell carcinoma were defined as partial responders.

Post-treatment MRI was compared with identical pretreatment MRI sequences. Studies were reviewed by an experienced head and neck radiologist (F.A.P.) and scored according to a 4-point grading scale introduced by Ojiri et al.[16] (see Table 1 for details and Figures 1-4).

In our study, 3 patients were excluded because of the presence of metastatic disease at a distant site just before the start of treatment. Six patients were excluded due to death before or just after completion of treatment caused by: pneumonia (n = 3), cervical spondylitis (n = 1), ruptured abdominal aneurysm (n = 1) and arterial (carotid artery) bleeding (n = 1). Other reasons for exclusion were lack of qualifying pretreatment (n = 4) and post-treatment (n = 10) MRI examinations. The above resulted in a final study population of 82 patients (61 males and 21 females). Tumors were staged according to the UICC guidelines[17]. The T and N-stage distribution were as follows: T3, 19; T4, 63; N0, 29; N1, 6; N2a, 1; N2b, 15; N2c, 25; N3, 6, resulting in 11 patients with stage III and 71 patients with stage IV disease. Primary tumor volumes were measured on MRI[18] and ranged from 6.4 cm^3 to 393.0 cm^3 (mean 41.8 cm^3, median 32.6 cm^3). The distribution of sites was as follows: oral cavity (n= 20), oropharynx (n= 52), hypopharynx (n= 7) and supraglottic larynx (n= 3). Patient's characteristics are summarized in Table 2.

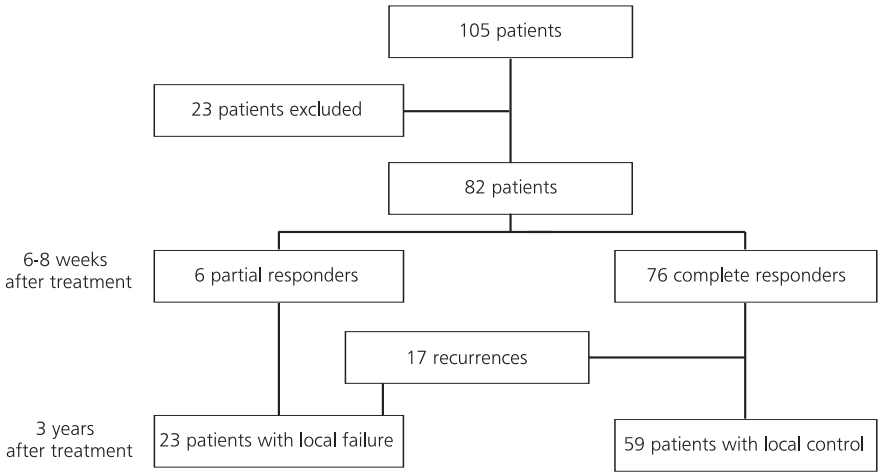


FIGURE 1. Flowchart of the outcome of the patient population.

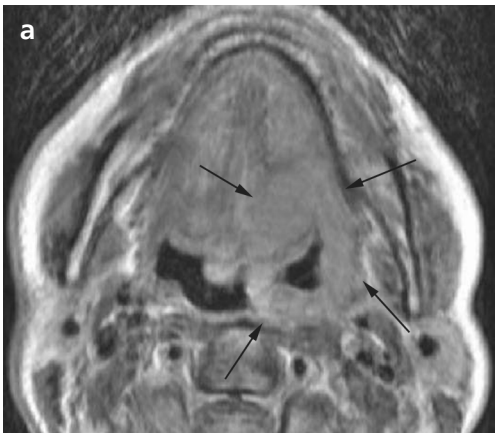
TABLE 2 Patient population

Variable	N	(%)
Gender		
Male	61	75
Female	21	25
T- classification		
T3	19	24
T4	63	76
N- classification		
N0-N1	35	42
N2-N3	47	58
TNM-stage		
Stage III	11	14
Stage IV	71	86
Site		
Oral cavity	20	24
Oropharynx	52	63
Supraglottic larynx	3	3
Hypopharynx	7	10
Infusion mode		
Unilateral intra-arterial	40	49
Bilateral intra-arterial	42	51

Patients were subjected to regular outpatient follow-up with chest X-ray routinely performed each year. All patients that completed treatment and survived had a minimum follow-up period of three years.

Statistical methods

Local failure was defined as residual disease after treatment or recurrent disease at the primary site during follow-up. Probability of local control was calculated by using the Kaplan-Meier method. Significance of differences in local control was analyzed by means of the log-rank test. For calculation of sensitivity, specificity, positive and negative predictive values of post-treatment MRI, the local response at three years was used. Post-treatment MRI findings were analyzed in combination with a pretreatment probability model, which has been published earlier in this journal[18]. The two variables, post-treatment MRI findings and pretreatment predictive factors (primary tumor volume, uni/bilateral intra-arterial infusion, T-classification, N-classification, age and site), were implemented in the final multivariable analysis, to determine their independent predictive value. In this multivariable analysis Cox proportional hazard model[19] was used and the variable 'posttreatment MRI findings' was split into negative and positive findings. Since MRI grade 1 only represents minor changes, patients with MRI findings grade 0 or 1 were defined as MRI-negative. MRI grade 2a or 2b were defined MRI-positive. The procedure PROC PHREG (SAS system for Windows release 8.02) was used to perform the multivariable analysis.



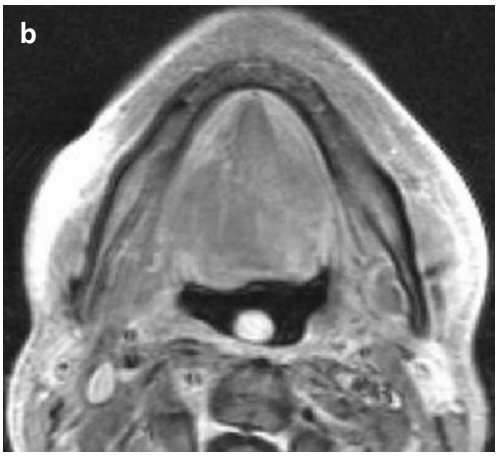
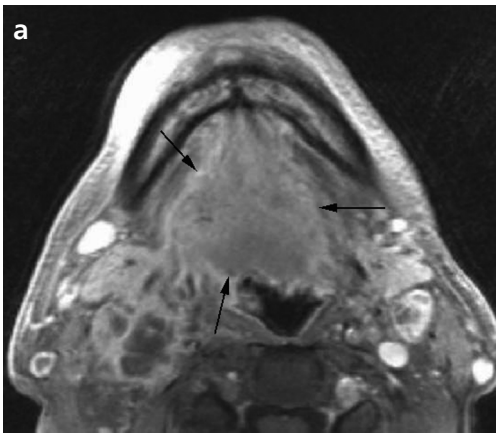


FIGURE 2. Example of grade 0 posttreatment MRI. (a) Contrast enhanced, pretreatment MRI image (T1-weighted) at tongue base level showing an advanced primary oropharyngeal tumor extending to the lateral and posterior pharyngeal wall (arrows). (b) Contrast-enhanced, posttreatment MRI image (T1-weighted) of the same patient at the same level as figure 1a, showing no detectable focal abnormalities, which was graded 0. Biopsies taken 8 weeks after treatment were negative. The patient remained disease free during a 5-year follow-up period.
Note: bilateral necrotic lymphadenopathy in level II.



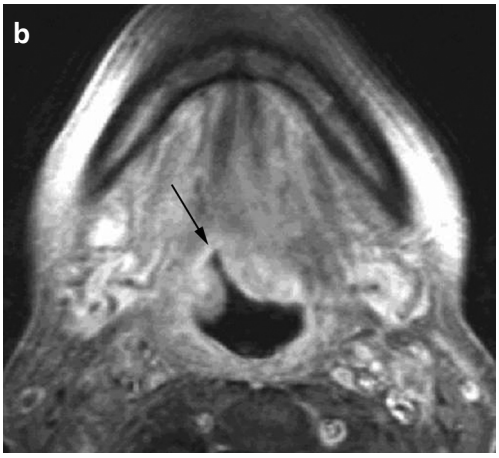


FIGURE 3. Example of grade 1 posttreatment MRI. (a) Contrast enhanced, pretreatment MRI image (T1-weighted) centered in floor of mouth showing the primary tumor (arrows). (b) Contrast-enhanced, posttreatment MRI image (T1-weighted) of the same patient at the same level as figure 2a, showing anatomic asymmetry (arrow), which was graded 1. Biopsies taken 6 weeks after treatment were negative. The patient remained disease free during a 3.5-year follow-up period.
Note: bilateral residual lymphadenopathy

RESULTS

In this study group of 82 patients, biopsies were taken in 42 cases. Biopsies from 6 patients (14%) collected during EGA, contained squamous cell carcinoma, which resulted in an initial local response rate of 93%. After a median follow-up of 29 months (range 2 - 71 months), 23 of 82 patients (28%) had a local failure (figure 1). Salvage surgery was performed in 5

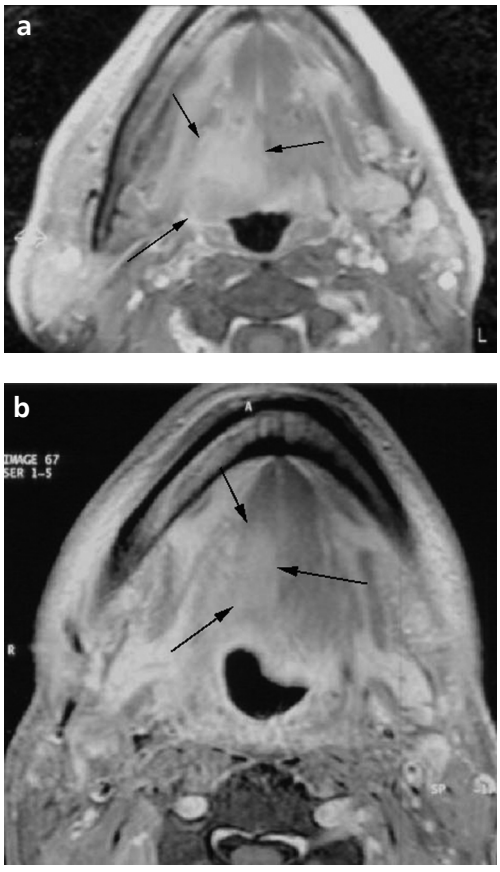


FIGURE 4. Example of grade 2a posttreatment MRI. (a) Contrast enhanced, pretreatment MRI image (T1-weighted) at the level of the tongue base showing the primary oropharyngeal tumor measuring 40 x 29 mm. (b) Contrast-enhanced, posttreatment MRI image (T1-weighted) of the same patient at the same level as figure 3a, suggesting a residual mass of 19 x 15 mm, which was graded 2a. Biopsies taken 8 weeks after treatment were negative. The primary tumor did not recur during a 3-year follow-up period.

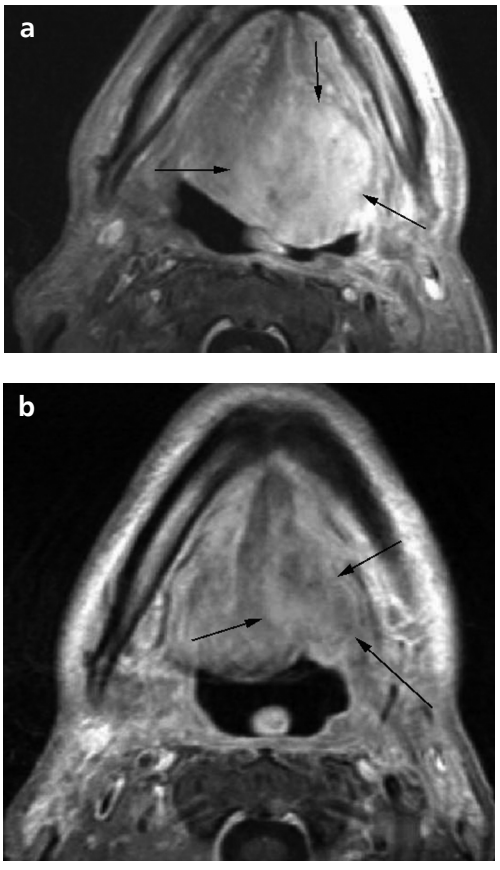


FIGURE 5. Example of grade 2b posttreatment MRI. (a) Contrast enhanced, pretreatment MRI image (T1-weighted) at the level of the tongue base showing the primary oropharyngeal tumor (arrows) measuring 53 x 42 mm. (b) Contrast-enhanced, posttreatment MRI image (T1-weighted) of the same patient at the same level as figure 4a, showing a discrete mass (arrows) of 31 x 23 mm, which was graded 2b. Biopsies taken 7 weeks after treatment showed squamous cell carcinoma.

patients one of them was still alive at the end of the study. The other four patients died within 6 months after salvage surgery. At the end of the study 30 patients had died of disease and 15 had died of other causes.

Posttreatment MRI findings (Table 3) were as follows: grade 0, 47 patients; grade 1, 15 patients; grade 2a, 16 patients, and grade 2b, 4 patients. Calculated sensitivity, specificity, positive and negative predictive values for posttreatment MRI were 48%, 85%, 55% and 81%, respectively (Table 4). After 3 years, the following recurrences were observed in the MRI posttreatment groups: grade 0, 9/47 recurrences; grade 1, 3/15 recurrences; grade 2a, 7/16 recurrences and grade 2b, 4/4 recurrences. Twelve out of 62 patients (19%) with

TABLE 3 Clinical outcome and posttreatment MRI findings at primary site

	EGA*			
MRI findings	**Negative**	**Positive**	**local failure****	**local control****
Grade 0	46	1	9	38
Grade 1	15	0	3	12
Grade 2a	14	2	7	9
Grade 2b	1	3	4	0

* Evaluation under general anesthesia, 6-8 weeks after treatment
**Three years after treatment

TABLE 4 Diagnostic accuracy of MRI

	Sensitivity	**Specificity**	**PPV**	**NPV**
MRI	48%	85%	55%	81%

TABLE 5 Cox proportional hazards analysis for local control

Variable	**p-value**	**Hazard Ratio**	**Hazard Ratio 95% Confidence Limits**
pretreatment factors	0.0010	2.384	1.422 - 3.998
posttreatment MRI	0.0137	2.968	1.249 - 7.052

Posttreatment MRI has been analyzed in combination with a prediction model consisting of pretreatment factors which has been described earlier[17]

MRI-negative findings (grade 0-1) had a local recurrence within 3 years after the treatment was completed. In only one of these patients a histopathological proof of residual disease was found during posttreatment EGA. Of the patients with a positive MRI, two out of 7 local failures within the MRI grade 2a group were detected during posttreatment EGA. Three out of four grade 2b patients were partial responders. The remaining patient had a local recurrence 11 months after completion of therapy. Figures 2-5 show examples of the posttreatment MRI with the matching pretreatment MRI as a baseline image.

A Kaplan-Meier plot demonstrates significant difference between curves stratified for negative and positive posttreatment MRI findings (log rank, $p < 0.001$, Figure 6). After the multivariable analysis, posttreatment response measurement by MRI emerged as an independent prognostic parameter for local control (hazard ratio 3.0, $p = 0.01$, Table 5).

In 42 cases biopsies were taken, because a suspicion of residual disease existed. In 10 out of 23 patients with local failures, no biopsy was taken at the initial EGA, because of absence of any suspicious findings. Fourteen of 82 patients had persistent ulcers, all of whom had negative biopsies 6-8 weeks after treatment. After 3 years, 4 (29%) out of these fourteen patients had a tumor recurrence.

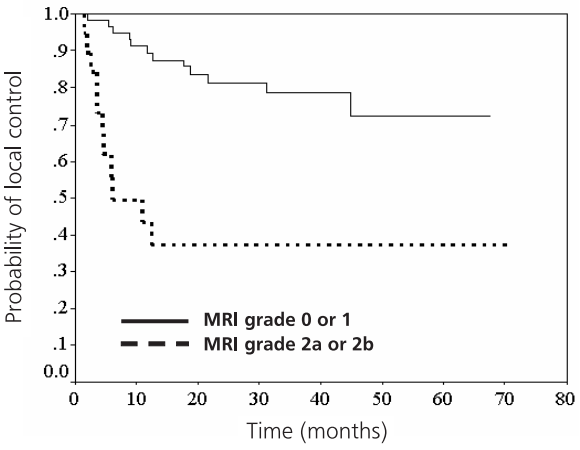


FIGURE 6. Kaplan-Meier curves of patients, stratified for posttreatment MRI findings: grade 0 and 1 (——) and grade 2a and 2b (·····). Log-rank test: $p < 0.001$. Grade 0 and 1 MRIs demonstrate no detectable focal abnormalities, anatomic asymmetry or a discrete mass ≤ 10 mm. MRIs were scored 2a or 2b if a discrete mass > 10 mm was detectable.

DISCUSSION

Adequate radiological measurement of tumor response after chemoradiation is important. MRI is a useful tool, since it delivers superior soft tissue contrast and exact delineation of tumor margins in arbitrary planes. Since postchemoradiation cross-sectional studies often demonstrate anatomic asymmetries[20,21], harboring the risk of false positive outcomes, appropriate judgment by an experienced head and neck radiologist remains a prerequisite to reduce the number of false positive results. This may explain the relatively high specificity of 85% in our series, particularly when compared to the earlier published percentages of 41%[22] and 44%[23] in patients treated with neoadjuvant chemoradiotherapy. The consistent implementation of pretreatment MRI in the final judgment of response measurement may also add to this striking difference in specificity. In this manner, Hermans et al.[11] were able to achieve a specificity of 95% in detecting local failures of laryngopharyngeal carcinomas in patients treated with only radiotherapy. The sensitivities presented in the studies of Kitagawa et al.[22] and Kubota et al.[23] were 100% and 75% respectively. The relatively high value demonstrated by Kitagawa et al.[22] might be caused by the low number of local failures (17%, n= 4) and the short evaluation period after treatment (within 6 weeks).

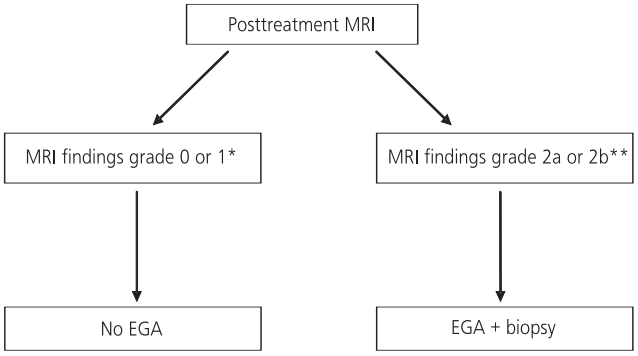


FIGURE 7. Flowchart for patients treated with chemoradiation for posttreatment response measurement. EGA = evaluation under general anesthesia
*Only limited changes or focal masses ≤ 10 mm.
**Discrete mass > 10mm.

Evaluation under general anesthesia (EGA) ± biopsy for assessment of residual disease in all chemoradiation patients following posttreatment MRI has limited value. Despite the fact that eventually 12 out of the 62 patients with MRI negative findings developed a local recurrence, only one of these was detected and proven histopathologically during the initial EGA. Since the probability of local failure in patients with a positive MRI is higher (55%) than in patients with a negative MRI (19%; $p<0.001$, Figure 5), EGA plus biopsy should be limited to patients with grade 2a and 2b. One could consider that EGA by MRI guidance might introduce a bias. Since biopsies were only taken when the MRI was abnormal or when the area of interest showed any visual or palpable findings suspicious for residual disease, the possibility exists that this might have caused an advantage in favor of EGA to detect residual disease. Without MRI less biopsies should have been taken. We recommend to implement the findings of this study in daily clinical practice (see algorithm, Figure 7) to increase the detection rate of local failures and to avoid unnecessary biopsies during EGA, which may put the patient at risk for osteoradionecrosis and/or postchemoradiation ulcers followed by arterial bleedings[24]. Because scoring is observer dependent, an experienced head and neck radiologist should perform assessment of MRI.

A well-known problem after chemoradiation is a persistent ulcer, which makes differentiation from residual disease difficult. In our study 14 patients had persistent ulcers (17%), of whom only four (29%) developed a local recurrence during follow-up. This percentage is not different from patients without ulcerative changes, meaning that extra biopsies or complementary imaging are of no value.

Primary tumor volume has proven to be one of the most important predictive factors for local control in head and neck cancer patients treated either with surgery[25], radiotherapy[26-28] or chemoradiation[29]. Because larger primary tumor volumes might be related to larger abnormalities on post-treatment MRI, we performed a multivariable analysis to determine the significance of this post-treatment factor demonstrating that posttreatment MRI is an independent predictive factor for local control (*p*-value of 0.014).

Increasing evidence is emerging that FDG-PET may evolve as a better imaging tool for early detection of recurrences. Reported sensitivity and specificity percentages range from 88% to 100% and from 78% to 94%[30-32]. It must be emphasized that in the majority of these studies patients with clinical suspicion of recurrence were included. The optimal interval between treatment completion and imaging remains questionable if FDG-PET is used. Particularly since this modality often results in false positive results in infected areas like postchemoradiation mucositis of mucosal defects.

In conclusion, evaluation under general anesthesia with biopsies taken six to eight weeks after chemoradiation in patients with advanced, inoperable tumors for response measurement is not indicated in patients with only limited changes or focal masses ≤ 10 mm on posttreatment MRI evaluations. In addition to pretreatment predictors, posttreatment MRI provides superior predictive information on local control.

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CHAPTER 6

Effectiveness of selective and radical neck dissection for regional pathological lymphadenopathy after chemoradiation

Lisa van der Putten, Guido B. van den Broek, Remco de Bree, Michiel W.M. van den Brekel, Alfons J.M., Frank J.P. Hoebers, Patricia Doornaert, C. René Leemans, Coen R.N. Rasch

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ABSTRACT

BACKGROUND. Diagnostic evaluation of the regional status after concurrent chemoradiation for advanced head and neck cancer remains difficult and indications for a salvage neck dissection and its extent are not clearly defined.

METHODS. In a series of 540 patients there was suspicion of regional residual or recurrent disease after chemoradiation in 61 patients who underwent 68 salvage neck dissections and 68 patients who were considered unresectable. For the patients with salvage neck dissection, accuracy of ultrasound guided fine needle aspiration cytology (USgFNAC) was determined. Disease control in the neck, disease specific and overall survival and parameters that may have prognostic value for the outcome were evaluated.

RESULTS. Neck dissection specimens contained vital tumor in 26 (43%) patients. Of these, 13 had selective neck dissections and 13 (modified) radical neck dissections. USgFNAC had a sensitivity of 80% and specificity of 42%. Nine patients developed a regional recurrence after salvage neck dissection (5 located in contralateral neck). Five-year regional control and overall survival rates were 79% and 36%, respectively. Significant prognostic factors for overall survival were surgical margins, presence of vital tumor cells and extent of neck dissection in univariable and only surgical margins in multivariable analyses.

CONCLUSIONS. For evaluation of radiation treatment response USgFNAC has a low specificity. Considering the good regional control rate and the high rate of unnecessary neck dissections with a theoretical planned neck dissection strategy, we conclude that a careful observational strategy is worthwhile and safe.

INTRODUCTION

A combination of concurrent chemotherapy and radiotherapy has become the treatment of choice for many patients with advanced head and neck squamous cell carcinoma (HNSCC) with significantly improved locoregional control and overall survival compared to radiotherapy alone[1,2]. These patients often present with advanced disease in the neck. Treating these patients goes along with several dilemmas. One of these includes the decision whether a planned neck dissection, for pretreatment N2-3 disease in the neck, or a salvage neck dissection for residual disease in the neck should be performed[3]. Although post-chemoradiation planned neck dissection is routine in many institutions, it is often considered as 'overtreatment' since in the majority of neck dissection specimens no vital tumor is found[4-9]. Furthermore, it delivers additional morbidity to patients who have already been subjected to severe toxicity during chemoradiation[10-12]. As a consequence, there is a tendency to perform post-chemoradiation neck dissections only if indicated by post-treatment diagnostic (clinical, radiological and/or cytologic) evaluation of the neck[13-17].

The advantage of limiting neck dissection to patients with residual neck disease 6-8 weeks posttreatment is that overtreatment is reduced. However, chemoradiation interferes with a reliable assessment of regional response due to treatment induced fibrosis, necrotic lymph nodes without tumor and false cytological results. Particularly in patients with substantial nodal tumor volumes, differentiation between scarred and partially necrotic lymph nodes tissue and residual metastases can be difficult[3,18]. If the decision to perform a neck dissection has been made, the next dilemma is determined by the extent of the neck dissection that needs to be performed, since evidence is emerging that selective neck dissection (SND) may be sufficient if it is highly likely that it removes the metastatic process entirely[19,20].

Over the years, ultrasound guided fine needle aspiration cytology (USgFNAC) in combination with palpation and routine posttreatment CT or MRI has been used as diagnostic tools in our institutes to decide whether a salvage neck dissection is indicated. For untreated necks the reported accuracy of USgFNAC is high, but to our knowledge no previous studies are done to evaluate the accuracy of USgFNAC in the detecting of lymph node metastases after non-surgical treatment[21-25].

In the current study, we investigated the reliability of USgFNAC and the effectiveness and safety of our careful observational strategy and neck dissection as determined by regional control and overall survival. We also determined prognostic factors for outcome.

chapter 6

PATIENTS AND METHODS

From November 1996 until November 2005, 540 patients with advanced HNSCC were treated with concomitant chemoradiation in the Netherlands Cancer Institute and the VU University Medical Center. This retrospective study included patients who were treated by 5 different schemes of chemoradiation. Two-hundred-seven patients were treated according to the intra-arterial chemoradiation schedule consisting of four consecutive weekly selective

intra-arterial infusions of cisplatin (150 mg/m^2) followed by intravenous sodium thiosulfate rescue combined with simultaneous radiotherapy according to the RADPLAT protocol[26-28]. One-hundred-sixty-one patients were treated with concomitant intravenous administration of 3 x 100mg/m^2 on day 1, 22 and 43 and 119 patients with a low dose intravenous concomitant scheme (daily 6 mg/m^2 cisplatin, 20 courses)[29]. All patients were irradiated daily for 6-7 weeks to a total dose of 70 Gy (2 Gy per fraction, 5-6/week). Fifty-three patients were treated according to the EORTC 24954 trial[30], 24 with an alternating scheme (cisplatin 20 mg/kg and 5-FU 200mg/kg (i.v.) in week 1, 4, 7, 10; radiotherapy in week 2, 3, 5, 6, 8, 9, total dose 60 Gy) and 29 with a sequential scheme (cisplatin 100 mg/kg and 5-FU 1000 mg/kg i.v., 4 courses; followed by 7 weeks radiotherapy, total dose 70 Gy). Both sides of the neck were radiated in all patients, regardless of the lymph node status. Four hundred-eight patients had evidence of neck node metastases before treatment. Treatment evaluation was performed 6-8 weeks after completion of chemoradiation. To measure response to therapy the treatment results were evaluated by clinical examination, USgFNAC, magnetic resonance imaging (MRI) and/or computer tomography (CT). During routine regular follow-up, imaging or USgFNAC was repeated if indicated. Positive cytology was defined by at least strong suspicion of vital tumor cells in the smear (all other cytological findings, e.g. necrotic (tumor) cells, were defined as negative). Immunocytochemical staining was not used. All patients with suspicion of persistent or recurrent lymph node metastases and who were considered operable underwent a neck dissection (ND). Lymph node metastases were defined persistent when diagnosed within 3 months after chemoradiation. Median follow-up was 18 months (range: 0 to 98 months). Minimum follow-up after completion of the chemoradiation was 1 year or until the patient deceased. Neck dissections were performed 6 to 87 weeks after completion of chemoradiation.

The extent of the neck dissection was based on posttreatment clinical and radiological evaluation. A selective neck dissection (levels I-III or II-IV) was performed for removal of limited residual mass in the neck; a (modified) radical neck dissection for removal of multiple or extensive residual metastases.

Using follow-up and histopathological examination of the neck dissection specimen as reference standard, sensitivity, specificity, positive and negative predictive values and overall accuracy were calculated for USgFNAC. Two independent groups were compared using the Chi-square test. Regional control and overall survival were calculated by the Kaplan-Meier method from the day of the neck dissection. Univariable analysis (log-rank test) was performed to determine the predictive value of the following variables for regional control and overall survival: primary tumor site, pretreatment T-classification, pretreatment N-classification, chemoradiation schedule, extent of ND, pathological examination of the surgical specimen. A multivariable analysis (Cox regression, Wald test) was performed to determine independent factors for overall survival after concurrent chemoradiation. Statistical analyses were carried out using SPSS 14.0.

RESULTS

Unresectable recurrences / residual disease group

Of the 540 patients, 68 had an unresectable regional residue (n=40) or unresectable regional recurrence (n=28) after chemoradiation. Of these 68 patients, 18 had N3 disease. Twenty-three of the 28 patients with a regional recurrence were considered unresectable because they also had an unresectable local recurrence (n=15) or distant metastases (n=8). Three patients had an unresectable regional recurrence without local or distant disease. Two other patients were found inoperable because of a poor general physical condition. The interval between chemoradiation and presentation of the unresectable recurrence varied from less than 3 months (n=3), 3-6 months (n=4), 6-12 months (n=9), 12-24 months (n=9) to more than 24 months (n=3).

Pretreatment staging versus recurrences

The chance of developing a regional recurrence or persistent disease was 11% for a N0-N1 neck, and 32% for a N2-N3 neck (Table 1, Figure 1). Of the patients with a N0-N1 neck before treatment 7 (3.5%) eventually underwent a neck dissection, in comparison to 54 (16%) of the patients with a N2-N3 neck.

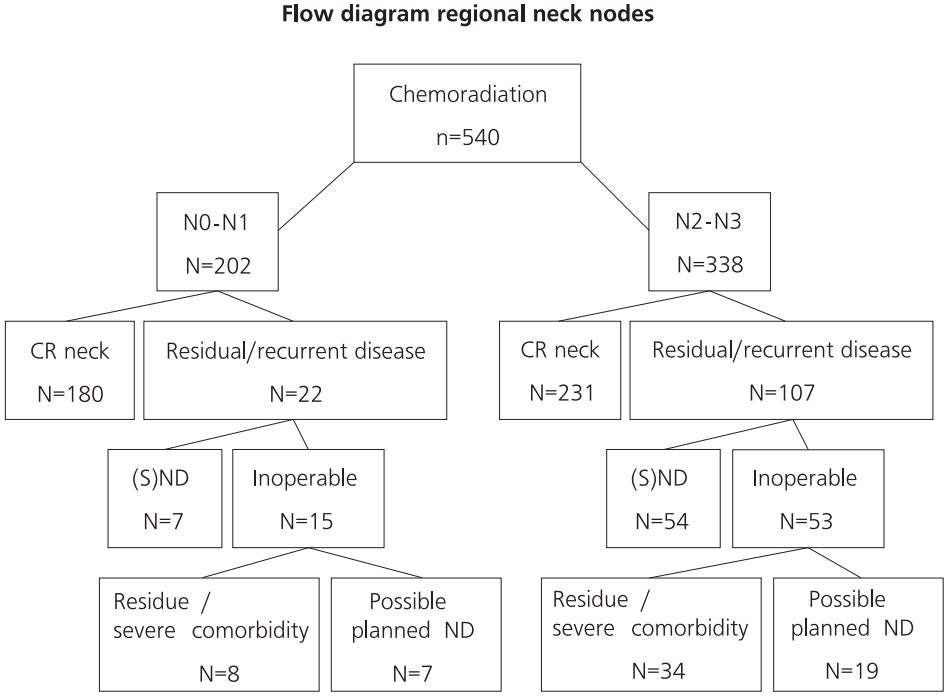

FIGURE 1. Flow diagram regional neck nodes.

TABLE 1 Classified per N-stage before chemoradiation: total number of patients with chemoradiation and number and percentage of regional residues and recurrences (total, with and without salvage neck dissection)

N-stage	Total (n)	Recurrences total (n)	(%)	Recurrences treated by ND* (n)	(%)	Recurrences not treated by ND* (n)	(%)
N0	132	6	4.5	3	2.3	3	2.3
N1	70	16	22.9	4	5.7	12	17.1
N2a	28	13	46.4	9	32.1	4	14.3
N2b	105	27	25.7	12	11.4	15	14.3
N2c	146	35	24.0	19	13.0	16	11.0
N3	59	32	54.2	14	23.7	18	30.5
Total	540	129	23.9	61	11.3	68	12.6

*Abbreviations. ND: neck dissection

Neck Dissection Group

In total, 68 neck dissections for residual or recurrent disease in the neck in 61 patients were performed: 42 selective and 26 (modified) radical neck dissections. The groups with SND and (M)RND did not show a statistical difference in pre-chemoradiation N-stage or diameter of the largest resected lymph node. The median interval between the last radiation and the salvage neck dissection was 14 weeks (range 6-87 weeks). After salvage surgery, the median follow-up period was 27 months (range 0-95 months). Patient's characteristics are summarized in Table 2.

Indications for neck dissections were a palpable mass and/or suspicion of persistent or recurrent lymph node metastases on MRI, CT or USgFNAC.

In 35 of the 61 patients (41 necks) no vital tumor was detected at pathology. In 25 of these, necrotic remnants of metastases were found. Residual or recurrent squamous cell carcinoma was histologically demonstrated in 26 patients (43%), i.e. 27 neck dissection specimens (14 SND; 13 (M)RND). In 23 of these 26 patients (88%) the metastases were found in the same levels as before treatment, representing the location of the largest lymph node metastases in 21 of these patients (81%). In 3 patients (12%) the metastases were found outside the original metastatic level as judged clinically and radiologically. All patients except one had residual or recurrent metastases in levels II-IV. Only one (2%) patient had a lymph node metastasis in level V. Thirteen SNDs (35%) and 13 (M)RNDs (54%) contained vital tumor, which was not a significant difference (Chi-square, p= 0.142).

At pathologic examination, negative margins were obtained in 75% of patients with a tumor containing SND and 62% of patients with a positive (M)RND (Trend test, p= 0.74). Of the 61 patients who underwent salvage neck dissection, three patients had residual disease after incomplete resection. Six others developed a regional recurrence after neck dissection. In only one of these 6 patients evidence for viable tumor cells had been found at histopathological examination of the previous neck dissection. In the other 5 patients only necrotic tissue was found in the neck mass. In 3 patients the regional recurrence was the only site of recurrent tumor whereas in the other 3 synchronously or metachronously a local tumor recurrence occurred.

In four of these nine patients, of whom three had an incomplete resection, the disease persisted or recurred in the operated neck. This concerned a histopathologic irradical (M)RND in 2 patients, an irradical SND which was found unresectable intra-operatively and a SND without tumor in the specimen but with a recurrence more than a year after the neck dissection. Five other patients developed a regional recurrence located in the contralateral neck, of whom 3 had originally a N2c neck with contralateral lymph node metastases ranging

TABLE 2 Patient characteristics of 61 patients with salvage neck dissection

Variables	Number	Percentage (%)
Gender		
male	51	84
female	10	16
Stage of disease		
III	4	7
IV	57	93
T-stage (before chemoradiation)		
T1	1	2
T2	8	13
T3	22	36
T4	30	49
N-stage (before chemoradiation)		
N0/1	7	11
N2a	9	15
N2b	12	20
N2c	19	31
N3	14	23
Primary tumor site		
oral cavity	6	10
oropharynx	34	56
hypopharynx	16	26
larynx	5	8
Chemoradiation schedule		
intra-arterial	27	44
intravenous	19	31
low dose	12	20
EORTC alternating	1	2
EORTC sequential	2	3
Ultrasound fine needle aspiration cytology		
positive	30	65
negative	16	35
Type of neck dissection		
selective neck dissection	42	62
radical neck dissection	26	38

from 0.9 to 1.0 cm. One of these 5 was treated with a contralateral neck dissection, the other 4 were unresectable.

Model of planned neck dissection strategy

To retrospectively estimate what the effect of a planned neck dissection strategy would have been, we developed a model. Patients with unresectable residual disease or a poor general physical condition could not have undergone a planned neck dissection, and were therefore excluded from the calculations (42 patients). The other patients with unresectable recurrences might have benefited from a planned neck dissection, just as the patients with an incomplete resection during salvage neck dissection (with neck dissections 4 to 9 months after chemoradiation). This group with possible benefit from a planned neck dissection strategy constitutes 5.8% of all patients (29/498); 5.2% in the N0-N1 group and 6.2% in the N2-N3 group (Figure 1). The percentage of unnecessary neck dissections (regional disease free patients) would have been 82.5% for the total group (411/498) and 92.8% and 76% for the N0-N1 and N2-3 group, respectively. For the patients who underwent a neck dissection (incomplete resections not included) for a regional residual disease or recurrence (11.7% of the total group (58/498); 2.0% N1-N2, 17.8% N2-N3) no clear benefit or disadvantage was found retrospectively.

USgFNAC

Of the 61 patients, positive USgFNAC results were achieved in 30 and negative results in 16 patients. In 15 patients no USgFNAC was performed as the clinical or imaging evidence of metastasis was convincing.

Twelve (40%) of the 30 patients with positive USgFNAC had histologically proven residual disease in the neck dissection specimen (true positive). Three USgFNAC's (19%) out of 16 negative aspirates were false negative. The sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of USgFNAC were 80%, 42%, 40%, 81% and 57%, respectively (Table 3). There was no significant relation between the time interval between radiotherapy and USgFNAC and the presence (mean 15.5 weeks) or absence (mean 14.8 weeks) of vital tumor in the neck dissection specimen (Independent T-test, p= 0.91).

TABLE 3 Diagnostic accuracy of USgFNAC*

			Pathology neck dissection		
			+	-	total
USgFNAC*		+	12	18	30
		-	3	13	16
		total	15	31	46
	Sensitivity	**Specificity**	**PPV***	**NPV***	**Overall accuracy**
USgFNAC*	80%	42%	40%	81%	57%

*Abbreviations. USgFNAC: ultrasound guided fine needle aspiration cytology, PPV: positive predictive value, NPV: negative predictive value

Survival and Regional Disease Free Survival

Of the whole patient group of 540 patients, after chemoradiation 76% (411/540) remained free of regional disease during follow-up (5-year regional control rate of 71%, analyzed from the last radiation date). The 5-year regional control from the date of salvage neck dissection for the ND study group of 61 patients was 79%. The 5-year regional control was 77% after SND and 90% after (M)RND, but this difference was not statistically significant (log-rank test, p= 0.70). All other variables were not predictive for regional control as well. Therefore a multivariable analysis was not performed.

The 5-years overall survival for the total study group was 36% (Figure 2). Univariable analysis demonstrated that the type of ND (log-rank test, p=0.04), histological demonstration of viable squamous cell carcinoma in the neck dissection (log-rank test, p= 0.03, Figure 3) and surgical margins (log-rank test, p< 0.001) were significant prognostic factors for overall survival (Table 4). In a multivariable Cox-regression analysis with the variables: tumor viability, pretreatment T- and N-stage, type of ND, surgical margins and chemoradiation scheme, the surgical margins emerged as the only significant prognostic factor for overall survival (p<0.001, hazard ratio 0.098, 95%CI: 0.040-0.240). The presence of vital tumor cells and the type of ND both showed a trend when analyzed in the subgroup of patients with negative surgical margins, but lost their significance in multivariable analysis of the total group.

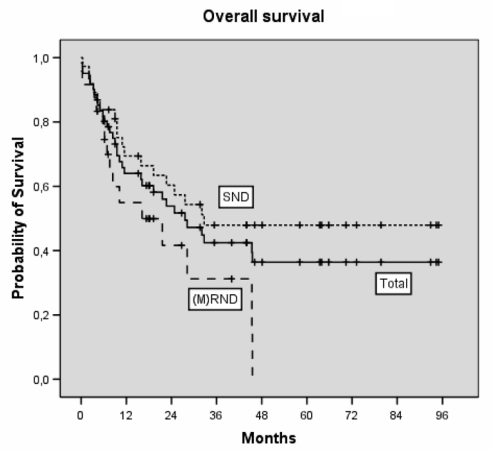

FIGURE 2. Overall survival of selective neck dissection (SND)-group, (modified) radical neck dissection ((M)RND)-group and all patients. Log- rank test comparing the SND- group and RND-group was statistically significant: p= 0.04.

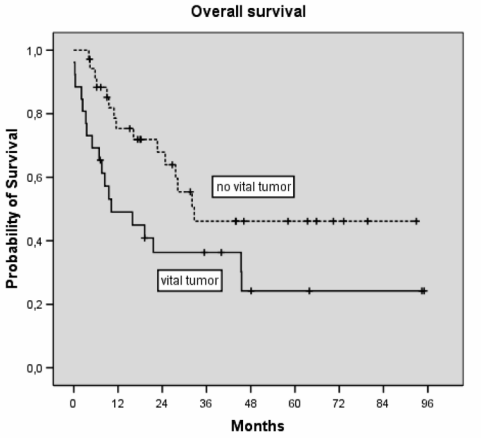

FIGURE 3. Overall survival of patients with vital tumor in specimen and patients without vital tumor in specimen. Log- rank test comparing both groups was significantly different: p= 0.03.

chapter 6

TABLE 4 Univariable analysis and multivariable analysis for overall survival

Variable	p-value	
	univariable	multivariable
T-stage	0.75	0.21
N-stage	0.33	0.33
Chemoradiation scheme	0.17	0.17
Tumor site	0.96	0.53
Tumor stage	0.70	0.29
Viable tumor	0.03	0.66
Type of neck dissection	0.04	0.25
Surgical margins	<0.001	<0.001

DISCUSSION

One of the major controversies today in head and neck oncology concerns the question of planned neck dissection or a salvage procedure after chemoradiation. Up till now there are no well designed randomised trials available which demonstrate the benefit of one of the two surgical approaches. A tendency of beneficial effects of planned neck dissection has been observed, but this finding is not supported by strong statistical significance[31]. Goguen et al.[32] retrospectively investigated 55 patients in a non-randomised way who underwent a neck dissection after concurrent chemoradiation. Patients with N2 disease had no benefit of a routine planned neck dissection. When patients with and without a neck dissection after a complete clinical response (indicated by physical examination and imaging studies) were compared, there was no survival benefit performing a planned neck dissection. Several years ago, Mendenhall et al.[33] reported that planned neck dissection was not needed for patients with N0-N1 disease who underwent radiotherapy or for patients with N2 disease, when chemotherapy was added to radiotherapy. This was confirmed by Argiris et al.[34]. They found better results for planned neck dissection only in patients with N3 disease or a salvage ND in patients without clinical complete response. These studies underline the importance to select patients for a ND, but it remains unclear whether N2 disease should be the cut-off point for planned surgery. In our study, pretreatment N-stage seemed to be correlated with the chance to develop a neck recurrence. Thirty-two percent of the patients with a N2-3 staged neck pretreatment had residual or recurrent regional disease, compared to 11% of the patients with a N0-1 staged neck. Richey et al.[35] reported that patients with initial N3 disease had a lower survival following attempted salvage surgery. We could not confirm this in the present study.

In total, 129 of 540 patients had a neck recurrence of whom 61 were suitable for a salvage neck dissection and 68 patients were considered unresectable at the time of decision for surgery. In 42 of these 68 patients a planned ND would not have prevented the neck disease to become unresectable because: a) the regional residual disease was unresectable at the end of chemoradiation or b) the patient was inoperable because of severe comorbidity. For the

other 26 patients it is difficult to assess whether they would have benefited from a planned neck dissection but in these patients early neck dissection could possibly have improved resectability. While early detection of 15 local recurrences in this group of 26 patients might have changed the treatment for patients with initially functionally unresectable disease, this would not have changed the treatment for technically unresectable disease. In 8 other patients distant metastases occurred at a later stage as well, and it is impossible to estimate whether these could have been prevented by early neck treatment.

When analyzed in our model, this resulted in a percentage of 6% who might have benefited from a planned neck dissection, while this planned neck dissection would have been unnecessary in 76% of the patients with N2-N3 disease. For patients with N0-N1 neck the number of unnecessary neck dissections is even higher (92.8%). Together with the relatively good regional control rate, this leads us to the conclusion that our watch and careful observational strategy has an acceptable outcome and that a planned neck dissection strategy would have resulted in a considererable overtreatment.

Although in this series a relatively good regional control rate is obtained, we realize that on the one hand probably still some unnecessary neck dissections were performed and on the other hand in some patients a delay has occurred by not performing a routine neck dissection. Especially in N2-3 disease, the chance of a regional residue or recurrence is over 30% and such a high risk should be considered in decision making. As a consequence, in these patients we now perform a neck dissection in case of doubt on the response in the neck at imaging or clinical examination. We do not recommend routine planned neck dissections in case of a complete response as this would result in a high percentage of unnecessary neck dissection.

Postchemoradiation evaluation

We evaluated the accuracy of USgFNAC and found a sensitivity of 80%, but a specificity of only 42%. USgFNAC used for the N-staging before treatment was reported to establish an accuracy of 86-97% with high specificity (83-100%)[21-24,36]. Knappe et al.[23] concluded that specificity was negatively influenced by misinterpretation of the smears by a less experienced pathologist. When the N0-neck was observed with USgFNAC during follow-up, a sensitivity of 50%-92% and a specificity that approaches 100% was found[25,37-39]. Considering the high specificity of USgFNAC when used for detection of lymph node metastases in untreated necks, it seems that the low specificity in this study is attributable to the effects of chemoradiation. To our knowledge, no other reports on this phenomenon are published.

The incidence of viable residual tumor cells in 43% of the neck dissection specimens is in agreement with the reported incidences in the literature, ranging from 29%-56%[3,18,40-42]. One should realize however that pathologists only look at a limited number of slides, with the consequence of missing viable micrometastases[43]. Moreover, the clinical meaning of finding only necrotic tumor cells is not known.

Increasing evidence exists that FDG-PET might be a valuable modality to evaluate response of the neck after chemoradiation. Brkovich et al.[13] and Porceddu et al.[44] found a high

negative predictive value of FDG-PET for diagnostic evaluation of the lymph nodes after chemoradiation. Results of PET are promising, but the accuracy seems to be dependent on the interval between treatment and imaging. A minimum interval of 8 weeks seems advisable[44-48].

Selective or modified radical neck dissection

The type of neck dissection is another difficult area of discussion. Although patient selection certainly played a role, in this series the overall survival was significantly better for patients with a SND (p=0.04) in univariable analyses, but this lost significance in multivariable analysis. This is most likely the result of a bias caused by pretreatment N-stage, although this was not statistically different for both groups. In the majority of cases a selective neck dissection was performed for removal of one residual mass. Robbins et al.[19] also reported a better regional control and overall survival for patients who underwent a SND compared to patients who underwent a RND. Recently he described promising results of superselective neck dissection for patients with persistent nodal disease confined to one level[46]. It seems therefore not necessary to perform a comprehensive ND in cases with limited residual or recurrent disease. However, the observed trend of better regional control after (M)RND (5-year regional control rate (M)RND 90%, SND 77%, p=0.70) indicates that decisions for selective neck dissection should still be made with caution. When it is unclear which neck levels are involved, a (M)RND is recommended.

Conclusion

In contrast to the high specificity in untreated necks, USgFNAC has a high number of false positives in patients treated with chemoradiation making this technique less reliable in these patients. More reliable detection techniques of regional recurrences are needed. Considering the good regional control rate in this study and the high rate of unnecessary neck dissections with a theoretical planned neck dissection strategy, we conclude that a careful watch and wait strategy is worthwhile and safe. Since the rate of residual or recurrent disease is high in N2-3 necks, in these patients we now perform a neck dissection in case of doubt on the response in the neck at imaging or clinical examination. In a selected patient group it seems safe to perform a selective neck dissection, although this decision should be made with caution.

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CHAPTER 7

Relationship between clinical factors and the incidence of toxicity after intra-arterial chemoradiation for head and neck cancer

Guido B. van den Broek, Alfons J.M. Balm, Michiel W.M. van den Brekel, Michael Hauptmann, Jan H. Schornagel, Coen R.N. Rasch

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ABSTRACT

BACKGROUND. Concomitant chemoradiation is more and more used for advanced head and neck cancer. It improves local control and survival compared to radiotherapy alone, but goes along with serious toxicity. This study was set up to determine the relationship between patient-, tumour-and treatment-related factors and acute/late toxicity after concomitant chemoradiation.

METHODS. One hundred and twenty-five consecutive patients with newly diagnosed inoperable stage III and IV head and neck cancer were enrolled for intra-arterial chemoradiation. There were 28 women (22%) and 97 men (78%) and the mean age was 55 years (range 30–80). One hundred and nine patients had stage IV disease (87%), 16 patients (13%) had stage III disease. Statistical analyses were performed to identify an association between factors and acute/late toxicity.

RESULTS. There were eight treatment-related deaths (6%). Severe acute toxicity (grade 3–4), mainly mucositis and dysphagia as categorized by the RTOG toxicity criteria, was recorded in 51% of the patients. Leucopenia (grade 3–4) occurred in 39% and aspiration pneumonia in 20% of patients. Tracheotomy was necessary in 15 (12%) patients. Neurological complications during treatment occurred in 3 (2%) patients. Severe late toxicity occurred in 34% of the patients. The most important of these were pneumonia (14%), osteoradionecrosis (9%) and swallowing problems with permanent percutaneous gastrostomy (20%). Statistical analysis did show a significant association between site and severe acute mucositis (p= 0.007), site and osteoradionecrosis (p= 0.014) and age and xerostomia (p= 0.004).

CONCLUSIONS. Chemoradiation is frequently associated with serious toxicity. Oral cavity tumours and older age are related to acute mucositis/osteoradionecrosis and xerostomia, respectively.

INTRODUCTION

Advanced head and neck cancer is increasingly treated with concomitant chemoradiation[1-6]. Better outcome is also reported in high-risk patients treated with surgery and postoperative chemoradiation compared to surgery and conventional postoperative radiation[7,8]. Nevertheless, this treatment is frequently associated with serious toxicity and treatment interruption[9,10]. Severe (grade 3–4) mucositis has been reported in approximately 50%[11] of chemoradiation cases and acute toxicities like nausea/vomiting, leucopenia, anaemia, renal dysfunction and dermatitis occur frequently as well. Late toxicity consists mainly of various degrees of swallowing problems, trismus, non-healing ulcers, osteoradionecrosis or loss of teeth and xerostomia, but detailed studies reporting late toxicity with a long follow-up remain limited. Because of the potentially life threatening characteristics of acute toxicities and the possible serious debilitating morbidity caused by late toxicity, prediction of toxicity is as important as prediction of treatment response and survival for patients with advanced head and neck cancer. This is especially important if alternatives, such as surgery with postoperative radiotherapy, are possible.

In 1992, Robbins et al. presented selective targeted chemoradiation for patients with advanced head and neck cancer consisting of 4-week consecutive (super-)selective intra-arterial infusions of cisplatin simultaneous with intravenous sodium thiosulfate rescue concurrent with radiotherapy[12]. This treatment gives the opportunity to lower systemic toxicity and increase chemotherapy dose. Meanwhile several intra-arterial chemoradiation studies have been reported[13-15] and outcomes seem not to be significantly different compared to intravenously delivered chemoradiation. However, systemic toxicity like leucopenia and gastrointestinal toxicity have been reported to be lower. A randomized trial comparing both infusion modes has to give definitive answers on these outcome and toxicity issues. We investigated the association between patient-, tumour- and treatment-related factors and acute and late toxicity in patients participating in phase II and III intra-arterial chemoradiation trials (RADPLAT) and undertook a review of published literature in order to assess and compare the incidence of acute and late toxicity in patients treated with concomitant intravenous or intra-arterial cisplatin and conventional radiotherapy.

PATIENTS AND METHODS

Patients

Between 1997 and 2002, 125 consecutive patients with newly diagnosed inoperable stage III and IV squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx and supraglottic larynx were enrolled for targeted chemoradiation. Except for hypopharyngeal carcinoma, all tumours were classified as surgically or functionally inoperable. Inclusion criteria were as follows: (1) oral cavity and base of tongue: no functional reconstruction possible after removal of the tumour, mainly including tumours requiring total glossectomy or resection of both hypoglossal nerves (2) tonsil and soft palate: extension towards the base of skull as manifested

by clinical trismus and apparent on imaging, making it highly unlikely to obtain clear surgical margins at the cranial border or requiring resection of the whole soft palate (3) posterior pharyngeal wall tumours or hypopharyngeal carcinomas: requiring total laryngectomy and extensive reconstruction, or fixation to the cervical spine (4) supraglottic larynx and/or base of tongue: tumour extensions requiring total glossectomy and total laryngectomy for complete removal. All statements regarding unresectability were reviewed by the two head and neck surgeons involved in this study (A.J.M.B., M.W.M.v.d.B.). A multidisciplinary team consisting of head and neck surgeons, radiation oncologists and medical oncologists initially evaluated all patients. Pretreatment work-up consisted of patient history, physical examination, blood tests (e.g. creatinine, haemoglobin level, leukocyte and platelet count and serum albumin level), examination under general anaesthesia, MR imaging and chest X-ray (or chest CT scan). Tumours were staged according to the UICC guidelines of 1997[16].

Treatment

All patients were included in a phase II (n = 79) or phase III (n = 46) trial: targeted intra-arterial chemoradiation (RADPLAT). Details on this treatment modality have been described earlier[13,15]. Briefly, treatment consisted of four consecutive weekly selective intra-arterial infusions of cisplatin (150 mg/m2) followed by intravenous sodium thiosulfate rescue combined with simultaneous radiotherapy according to the RADPLAT protocol[12] (2Gy per day, 5/week x 7 to a total dose of 70Gy). Twenty-four patients have been treated by intensity modulated radiation therapy (IMRT). IMRT was carried out to spare the salivary glands. In addition to the earlier reported intra-arterial administration of cisplatin, we performed bilateral infusion in patients whose primary tumours extended across the midline, with equal distribution of cisplatin doses over both sides. Patients were admitted during chemotherapy and were pre-hydrated 24 h before infusion. Prior to treatment, all patients signed an informed consent form approved by our Institutional Protocol Review Committee. Acute and late toxicity was prospectively assessed and registered on a standard toxicity form every 2 weeks during treatment and every visit (every 2–3 months) after treatment according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) and the Radiation Therapy Oncology Group (RTOG) toxicity criteria by radiation oncologists. Aspiration pneumonia was defined as a lung infection due to oral or gastric content with typical abnormalities on X-ray of the thorax. Dysphagia was assessed by interpretation of subjective symptoms of the individual patient.

Statistical analysis

Four toxicity outcomes were defined: grade 3–4 mucositis, osteoradionecrosis, grade 3–4 xerostomia and persistent tube feeding (longer than 2 months after treatment). We evaluated the association between the time from the first day of treatment to the time of the first occurrence of each outcome and the following treatment-related factors: size of radiotherapy field, unilateral vs. bilateral intra-arterial infusion; tumour-related factors: tumour volume, site, T-classification, N-classification, TNM-stage; and patient-related factors: age, gender, serum

albumin level and co-morbidity (ASA physical status (I: healthy, II: mild systemic disease, III: severe systemic disease); always assessed before pretreatment examination under general anaesthesia by the attending anaesthetist). Patients were censored at death or end of follow-up. Univariable Cox regression models were used to assess group differences. Patients with residual disease or recurrence were excluded from analyses for late effects if the recurrence occurred within 6 months of late toxicity. For descriptive purposes, we also produced Kaplan-Meier plots for local control and overall survival. Multivariable analyses of toxicities were not performed, because the number of severe toxicity events was relatively small (N < 15). Statistical analyses were carried out using SPSS 12.0.1.

RESULTS

The main characteristics of 125 patients are detailed in Table 1. Primary tumour volumes were determined in 60 patients, as described earlier[17]. The median tumour volume was 30 cm3 (range 6.9–86.3 cm3). Pretreatment laboratorial tests showed: median absolute leukocyte count 10.9 x 109/l (range 5.2–43.0), median absolute haemoglobin level 8.2 mmol/l (range 5.3–10.7), median creatinine level 0.76 mg/dl (range 0.28–1.30) and median serum albumin level 37 g/l (range 24–48, reference value 35–55 g/l). After a mean follow-up of 32 months (range 1–92 months) 29 patients had a local recurrence. Five of them have been salvaged surgically, but only one of these patients survived longer than 1 year. At the end of the study, 48 patients were alive, 45 patients had died of disease and 32 patients died of other causes. After 5 years, estimated local control rate and overall survival rate were 68% and 35%, respectively (Fig. 1).

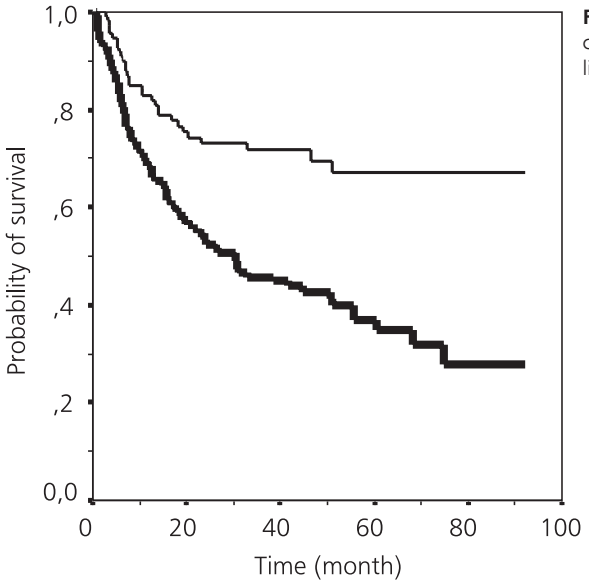

FIGURE 1. Kaplan-Meier estimates of local control (thin line) and overall survival (thick line).

TABLE 1 Patient population

Variable	N (%)
Gender	
Male	97 (78%)
Female	28 (22%)
T- classification	
T3	28 (22%)
T4	97 (78%)
N- classification	
N0-N1	48 (38%)
N2-N3	77 (62%)
TNM-stage	
Stage III	16 (13%)
Stage IV	109 (87%)
Site	
Oral cavity	24 (19%)
Oropharynx	76 (61%)
Supraglottic larynx	3 (2%)
Hypopharynx	22 (18%)
Co morbidity (ASA)	
1	42 (34%)
2-3	83 (66%)
Infusion mode	
Unilateral	58 (46%)
Bilateral	67 (54%)
Radiotherapy	
Conventional	101 (81%)
IMRT	24 (19%)
Size conventional RT field	
≤ 95 cm^2	65 (52%)
> 95 cm^2	36 (29%)

Acute toxicity

One hundred and thirteen patients (90%) fully completed their treatment schedule. Four patients, who did not complete their treatment, fulfilled at least three cycles of chemotherapy and received at least 64 Gy radiotherapy. The three reasons for treatment termination were tumour progression (n = 2), obstruction of the superior thyroid artery and a transient ischemic accident.

There were eight treatment-related deaths (6%), including sepsis after pneumonia in 5 patients, sepsis after renal failure in 1 patient, cervical spondylitis in 1 patient and 1 patient with a ruptured abdominal aneurysm. Exact acute toxicity data (according to RTOG criteria) of five patients were missing. The mean number of acute grade 3 and 4 toxicities per patient was 2.14 (median 2.00). Acute toxicities according to RTOG criteria are listed in Table 2 (n =

TABLE 2 Acute toxicity according to RTOG (N= 120)

Adverse Effect	Grade 1	Grade 2	Grade 3	Grade 4
Skin	57 (48%)	32 (27%)	15 (13%)	1 (1%)
Mucous membrane	10 (8%)	45 (38%)	59 (49%)	2 (2%)
Pharynx and oesophagus	12 (10%)	46 (38%)	53 (44%)	4 (3%)
Upper gastrointestinal tract	32 (27%)	41 (34%)	5 (4%)	0
Salivary gland	23 (19%)	33 (28%)	21 (18%)	0

Skin: 1= dry erythema, 2= patchy erythema, 3= confluent moist desquamation, 4= ulceration, necrosis
Mucous membrane: 1= mild pain, 2= patchy mucositis, 3= confluent mucositis, 4= ulceration, necrosis
Pharynx: 1= mild dysphagia, 2= moderate dysphagia, 3= severe dysphagia (N-G tube), 4= complete obstruction, perforation
Upper GI tract: 1-3 = anorexia with <5%(1), <15%(2) or >15%(3) weight loss from pretreatment baseline, 4= acute obstruction, perforation
Salivary gland: 1= no change, 2= mild mouth dryness, 3=moderate dryness, 4= does not exist, 5= acute salivary gland necrosis

120). Myocardial infarction occurred in 5 patients and 25 patients (20%) had a pneumonia. Forty-six patients (37%) suffered from grade 3 to 4 leucopenia. The peripheral leukocyte count dropped to the lowest level in the fifth week of treatment. During the course of treatment the following mean leukocyte counts were found: pretreatment 11.9 x 109/l, first week 11.2, second week 9.3, third week 5.5, fourth week 3.6, fifth week 3.3, sixth week 5.3 and seventh week 7.2. Forty-two (34%) patients needed blood transfusions because haemoglobin dropped below 6.0 mmol/l. Only one patient had a low haemoglobin level grade 3 in the fifth week of treatment and one patient suffered from grade 3 thrombopenia. Serious renal dysfunction only occurred in two patients (2%). One hundred and six (85%) patients had tube feeding and the mean duration of tube feeding was 8.42 (median 5.00) months. Thirty-eight (36%) patients started tube feeding before treatment and 68 (64%) patients started during treatment. Percutaneous gastrostomies were performed in seventy-five (60% of all patients) of these 106 patients. In five of these patients a gastric perforation occurred, which was successfully treated by surgery. Other toxicities, which occurred during or shortly after treatment, are listed in Table 3.
Intra-arterial infusion of cisplatin was performed 748 times and resulted in three patients with a neurological accidents (0.4%): transient ischemic attack (n = 1), cerebrovascular accidents (n = 2). All patients had a full neurological recovery with no rest symptoms after a couple of days. Local haematoma at the arterial puncture site (femoral artery) occurred in four patients.

Late toxicity

Late toxicities could be registered in 90 patients and are listed in Tables 3 and 4. Radiation induced toxicity as recorded by the RTOG toxicity criteria was registered in 70 patients, because late toxicity was not registered in 20 patients. The mean number of late grade 3 and 4 toxicities per patient was 0.56 (median 0.00). Eighteen patients (20%) were still dependent on tube feeding 12 months after treatment. One patient still had a tracheotomy, which

TABLE 3 Other acute and late toxicity according to NCI-CTC criteria

Toxicity	N (%)
Acute (N= 125)	
Leucopenia (grade III)	46 (37%)
Febrile neutropenia (grade III)	20 (16%)
(Aspiration) pneumonia (grade III)	25 (20%)
Tracheotomy	15 (12%)
Myocardial infarction	5 (4%)
Haematoma after intra-arterial infusion	4 (3%)
Cerebrovascular accident/transient ischemic attack	3 (2%)
Erysipelas	3 (2%)
Abscess of the neck	3 (2%)
Renal (grade III)	2 (2%)
Pharyngitis	2 (2%)
Thrombosis	1 (1%)
Epilectic seizure	1 (1%)
Late (N= 90)	
(Aspiration) pneumonia	13 (14%)
Hypothyroidism	10 (11%)
Osteoradionecrosis	9 (10%)
Trismus	9 (10%)
Polyneuropathie	5 (6%)
Loss of teeth	4 (4%)
Arterial bleedings	3 (3%)
Orocutanous fistula	1 (1%)
Stenosis of the oesophagus	1 (1%)

TABLE 4 Late toxicity according to RTOG toxicity criteria (N= 70)

Adverse Effect	Grade 1	Grade 2	Grade 3	Grade 4
Skin	28 (40%)	7 (10%)	0	0
Mucous membrane	31 (44%)	25 (36%)	3 (4%)	3 (4%)
Oesophagus	26 (37%)	16 (23%)	17 (24%)	1 (1%)
Subcutaneous tissue	21 (30%)	21 (30%)	4 (6%)	0
Larynx	18 (26%)	9 (13%)	3 (4%)	0
Salivary gland	14 (20%)	36 (51%)	12 (17%)	0

Skin: 1= slight atrophy, 2= patchy atrophy, 3= marked atrophy, 4= ulceration
Mucous membrane: 1= slight atrophy, 2= moderate atrophy, 3= marked atrophy, 4= ulceration
Oesophagus: 1= mild fibrosis, 2= unable to take solid food, 3= severe fibrosis, liquid diet only, 4= fistula, perforation
Subcutaneous tissue: 1= slight induration, 2= moderate fibrosis, 3=severe induration, 4= necrosis
Larynx: 1= hoarseness, 2= moderate oedema, 3= severe oedema, 4=necrosis
Salivary gland: 1= no change, 2= mild mouth dryness, 3=moderate dryness, 4= does not exist, 5= acute salivary gland necrosis

was probably due to laryngeal oedema as a result of severe smoking. Osteoradionecrosis was seen in nine (10%) patients and four were treated by hyperbaric oxygen treatment and the other five patients were successfully treated by surgery preceded by hyperbaric oxygen treatment. During follow-up, 14 (16%) patients suffered from pneumonia, which was effectively treated by intravenous administration of antibiotics in 13 patients. One patient died of pneumonia. Arterial bleeding from the primary site occurred in three (3%) patients, who were all successfully treated by embolization.

Univariable analyses were performed for severe (acute) mucositis, severe (late) xerostomia, persistent tube feeding and osteoradionecrosis as endpoints. All patients with a tumour recurrence were excluded from this analysis, because late toxicity occurred less than 6 months before the day of recurrence. Site was associated with severe mucositis, p= 0.007 and osteoradionecrosis, p= 0.014 (Table 5). The incidence of mucositis was significantly higher in patients with oral and oropharyngeal tumours compared to patients with hypopharyngeal and laryngeal cancer. Twenty-seven percent of patients with oral cancer developed osteoradionecrosis compared to 7% in patients with cancers at other sites. A statistically significant, positive association was noted for age and severe xerostomia, p= 0.004. Older patients were more sensitive for developing xerostomia, compared to younger patients.

TABLE 5 Univariable analysis of toxicity

Variables	Mucositis (p-value)	Xerostomia (p-value)	ORN* (p-value)	Tube feeding (p-value)
Age (≤ vs. > 55)	0.51	0.005	0.96	0.60
Gender	0.12	0.18	0.71	0.61
Co morbidity (ASA 1 vs. 2-3)	0.07	0.96	0.06	0.11
Serum albumin (≤ vs. > 37 g/l)	0.23	0.06	0.47	0.15
T-classification (T3 vs. T4)	0.84	0.95	0.65	0.07
N-classification (0-1 vs. 2-3)	0.55	0.06	0.47	0.04
TNM stage (III vs. IV)	0.75	0.92	0.49	0.21
Site	0.03	0.33	0.006	0.39
Tumour volume (≤ vs. > 30 cm^3)	0.28	0.47	0.39	0.23
Infusion mode (uni- vs. bilateral infusion)	0.12	0.24	0.38	0.70
Size of RT field (≤ vs. > 95 cm^2)	0.79	0.55	0.69	0.63

ORN= osteoradionecrosis

TABLE 6 Overview of literature: acute and late toxicity after concomitant cisplatin-based chemoradiation

Authors	Number of patients	LRC rate (5y)	OS rate (5y)
Intravenous CRT			
Homma et al.[21]	41	46%	36%
Jeremic et al.[5]	53	42%	32%
Bartelink et al.[18]	24	20%	13%
Glaser et al.[20]	36	45%	30%
Franchin et al.[19]	21	10*	16*
Marcial et al.[22]	124	43% (4y)	34% (4y)
Adelstein et al.[1]	95	45%	28%
Intra-arterial CRT			
Homma et al.[14]	43	69% (3y)	54% (3y)
Robbins et al.[15]	213	74%	39%
Balm et al.[13]	79	68%	43%
this series	126	65%	40%

[21] intra-venous cisplatin (4mg/ m2/ day/ 4weeks) and radiotherapy (2.0 Gy/ day/ 5days/ 6.5 weeks)
[5] intra-venous cisplatin (6mg/ m2/ day/ 7weeks) and radiotherapy (2.0Gy/ day/ 5days/ 7weeks)
[18] intra-venous cisplatin (35mg/ m2/ x6) and radiotherapy (1.8Gy/ day/ 5days/ 7weeks)
[20] intra-venous cisplatin (50mg/ m2/ x3) and radiotherapy (1.8Gy/ day/ 5days/ 7weeks)
[19] intra-venous cisplatin (100mg/ m2/ x3) and radiotherapy (1.8-2.0Gy/ day/ 7weeks)
[22] intra-venous cisplatin (100mg / m2/ 3x) and radiotherapy (2.0Gy/ day/ 5days/ 7weeks)
[1] intra-venous cisplatin (100-120mg/ m2/ week) and radiotherapy (2.0 Gy/ day/ 5days/ 6.5 weeks)
[13-15] intra-arterial cisplatin (150mg/ m2/ 4x) and radiotherapy (2.0Gy/ day/ 5days/ 7weeks)
NR= not reported
*= median time to progression, median survival time

DISCUSSION

For the last decades chemoradiation has developed as the standard treatment for patients with advanced, inoperable head and neck cancer[1-6]. Optimal treatment schedules have not yet been defined and are still the subject of many phase II and III trials. A thorough knowledge of accompanying acute toxicities is of utmost importance, since chemoradiation puts a heavy burden on the patient's condition. As there is a tendency to use this treatment as an organ and function preserving modality in operable advanced disease as well, it is valuable to be able to predict long term treatment morbidity. The aim of this study was to determine the relationship between factors and acute/late toxicity in patients treated with concomitant chemoradiation. This series includes 125 patients and acute toxicity was comparable with other published series[1,5,13-15,18-22](Table 6). Possible predictive factors for severe mucositis, severe xerostomia, persistent tube feeding and osteoradionecrosis were analyzed in a univariable manner. To our knowledge, this is the first report demonstrating the relationship between factors for toxicity other than treatment-related factors in head and neck cancer patients

Acute toxicity				Late toxicity
Mucositis (III-V)	**Leucopenia (III-IV)**	**Haematological toxicity (III-V)**	**Renal toxicity (III-V)**	**Total late toxicity (III-V)**
17%	NR	2%	0%	5%
13%	8%	11%	2%	4%
33%	38%	42%	NR	NR
NR	NR	6%	0%	NR
52%	NR	NR	NR	8%
31%	11%	NR	6%	NR
45%	42%	NR	8%	NR
37%	28%	35%	0%	NR
26%	8%	8%	0%	NR
43%	38%	38%	0%	NR
49%	37%	37%	2%	34%

treated with concomitant chemoradiation. Age was related to late xerostomia: older patients were significantly more likely to develop xerostomia than younger patients (p= 0.004). Site was predictive for severe mucositis (p= 0.007) and osteoradionecrosis (p= 0.014).

Cisplatin has been used in various doses and has been administered both intravenously and intra-arterially. Table 6 gives an overview of studies describing toxicities in patients treated with conventional radiotherapy (70 Gy) in combination with cisplatin and indicates that toxicities in patients who had intravenous or intra-arterial infusion of cisplatin seem comparable. In addition, all toxicities except mucositis incidence were similar in patients with different doses of cisplatin. Severe (grade 3–4) mucositis has been reported in 13–52% of all patients, who underwent cisplatin-based chemoradiation. In our study, acute mucositis (grade 1–4) was seen in almost every patient (97%) and severe mucositis (grade 3–4) was seen in 61 (51%) patients, which is comparable with other chemoradiation studies[11]. It seems that severe mucositis (grade 3–4) is associated with chemotherapy dose. In low dose chemotherapy schedules only 13–17% of all treated patients developed severe mucositis[5,21], whereas the incidence in normal and high dose treatment schedules was between 26% and 52%. Other studies have demonstrated differences in toxicity to be related to various treatment schedules[9,11]. Trotti et al.[11] undertook a systematic review and demonstrated that mucositis is more common in patients treated with altered fractionation radiation (100%) than in chemoradiation patients (89%). In addition to that, severe mucositis (grade 3–4) is also more common in patients treated with altered fractionation radiation compared to chemoradiation patients, 56% and 43%, respectively. In our series, patients with a tumour located in the oral cavity and oropharynx experienced significant more mucositis than patients with tumours of other sites. One could consider that the oral cavity is easier to examine than the larynx or

hypopharynx and this might have introduced a bias. Another explanation could be the large surface of mucosa in the oral cavity, which is affected during chemoradiation. A validation study should confirm these data.

Apart from mucositis, chemoradiation is associated with several other acute toxicities, often necessitating intensive patient care and prolonged hospitalization. Due to intensive patient care, the dropout number in this series could be kept relatively low: 90% of patients completed their treatment schedule. One hundred and six (85%) patients had tube feeding before or during treatment. Since weight loss is a prognostic factor for survival[17,23], it is important to ensure sufficient intake, either orally or by tube feeding. As a result, tube feeding was provided in patients with >10% weight loss or patients who had insufficient oral intake. In thirty-eight (36%) patients tube feeding was started before treatment. These patients did thus not have tube feeding because they suffered of mucositis or dysphagia as a result of chemoradiation-treatment. Percutaneous gastrostomy was performed in patients who needed tube feeding for a period longer than 6 weeks. Consequently, all patients who started tube feeding before and in the beginning of treatment (n = 75, 60%) underwent percutaneous gastrostomy. This clarifies the relatively high number of patients with tube feeding and percutaneous gastrostomy. Older patients were significantly more likely to develop xerostomia than younger patients. In the literature, xerostomia is usually not related to age in patients treated with radiotherapy. Intensity modulated radiation therapy (IMRT) was mostly used in younger patients and could be an explanation for this outcome. However, statistical analysis (not shown) could not confirm this hypothesis.

Severe haematological toxicity was seen in 47 patients. Most of these patients (n = 46) suffered from leucopenia. Intra-arterial chemotherapy infusions were delivered in the first 4 weeks of treatment, which resulted in the lowest leukocyte levels in the fifth week of treatment. Comparing different treatment schedules, no difference is observed in incidence of leucopenia.

Treatment-related deaths did occur in 6% of patients, including sepsis after pneumonia in five patients, sepsis after renal failure in 1 patient, cervical spondylitis in 1 patient and 1 patient with a ruptured abdominal aneurysm. Most of these deaths were related to toxicity of chemoradiation and not related to the mode of infusion. The rupture of the abdominal aneurysma might be induced by an intra-arterial infusion, although the rupture was a couple of days after the infusion. Neurological complications induced by intra-arterial infusions were scarce: only two cerebrovascular accidents and one transient ischemic attack occurred in 748 infusions (0,4%). It is likely that this low complication rate seems to be related to expertise of the individual intervention radiologist. Patients with a history of cerebrovascular accident were excluded for intra-arterial chemoradiation. Other late toxicity problems that are associated with concomitant chemoradiation and often necessitating intensive care and prolonged hospitalization are persistent tracheotomy (1 patient) and arterial bleedings (3 patients). The arterial bleedings had a sudden onset and occurred approximately 1 year after treatment. All bleedings were successfully treated by embolization.

Conclusion

Chemoradiation is associated with severe toxicity in the majority of patients, necessitating intensive care of these patients. There were eight treatment-related deaths (6%). Mucositis is the main problem during treatment and is present in the majority of patients, mainly those with oral and oropharyngeal cancer. Swallowing problems, pneumonia and osteoradionecrosis are the most prominent late toxicities. We realize that multiple testing might increase the Type I error rate, but analysis of 11 variables demonstrated well understandable associations between site and acute mucositis/ osteoradionecrosis and age and xerostomia. Although these factors are not modifiable and probably not useful for patient selection, these data can be used for anticipation of problems in patient' care and providing information for patients. Reviewing the literature, small differences in toxicity (mucositis, systemic toxicity) between intra-arterial and intravenous chemoradiation schedules were observed. A randomized phase III trial comparing both infusion modes was recently closed and has to give definitive answers on these toxicity issues.

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CHAPTER 8

Summary and conclusions / Samenvatting en conclusies
(Dutch Summary)

SUMMARY

Chapter 1

In this chapter the background of non-surgical treatment of advanced head and neck cancer is described with emphasis on the development of concurrent chemoradiotherapy (CCRT). This treatment was developed as an organ sparing treatment option with curative intent for advanced head and neck cancer. Initial results with intra-arterial administration of high dose cisplatin (4x 150mg/m^2) in combination with radiotherapy (70Gy) were promising with high initial locoregional complete response rates. The aim of the thesis is to charcterize, clinically and biologically, patients who may benefit from intra-arterial chemoradiation. To identify these patients tumor-, patient- and treatment related variables were investigated for their predictive value using local control and overall survival as outcome measures.

Chapter 2

The predictive value of common clinical and radiological predictive factors, such as age, gender, co-morbidity, site of primary tumor, tumor volume and TNM stage were tested in 92 consecutive patients. Side of intra-arterial infusion of chemotherapy (unilateral vs. bilateral) was also added as a specific treatment related factor. Primary tumor volume and unilateral infusion influenced local control significantly. Using tumor volume as a continuous variable an adjusted risk ratio of 1.026, was found, indicating that each 1 cm^3 increase in volume is associated with a 2.6% decrease in probability of local control. Primary tumor volume, co- morbidity, lowest involved neck node level and pretreatment weight loss > 10% were significant predictors for poor overall survival. Primary tumor volume appears to be an essential variable for selecting patients for concomitant intra-arterial chemoradiation. A nomogram, based on the significant prognostic variables, was developed for clinical use.

Chapter 3

Chapter 3 describes the results of a comparative genomic hybridization (CGH) analysis in 40 patients treated with RADPLAT. Genetic alterations from 20 chemoradiation-sensitive and 20 chemoradiation-resistant patients were compared. Resistant tumors were characterized by a distinct profile of genetic changes: high level amplifications were more frequent. Gains of 3q11-q13, 3q21-q26.1 and 6q22-q27 and losses of 3p11-pter and 4p11-pter were significantly associated with chemoradiation resistance. High-level amplifications unique to resistant cases involved the chromosomal regions 1p32, 3q24, 7p11.1, 7p11.2-12, 8p11.1, 8p11.1-12, 12q15, 13q21, 15q12, 18p11.3 and 18q11. Although we were able to demonstrate significant differences in the genetic profiles, identification of possible molecular markers and analysis of their function is needed to explain the role of chromosomal alterations in chemoradiation resistance.

Chapter 4

This chapter describes the identification of candidate genes for chemoradiation resistance. Ninety one tumor biopsies of RADPLAT patients were evaluated for protein expression on a tissue micro-array (TMA). Statistical analysis showed significantly increased hazard ratios of RB, P16 and MRP2 for local control and P-glycoprotein and HIF-1α for overall survival. MRP2, P-glycoprotein and P16 levels were positively associated with outcome whereas RB and HIF-1α had a negative relationship. Although an association between all markers and local control was not demonstrated by Goeman's global test, an association between a combination of three markers (P16, P21, and P27) and outcome (p=0.05) was found. In a multivariable analysis only MRP2 and P16 were significant independent predictive markers, specifically patients with biopsies showing overexpression of MRP2 or P16 had an increased probability of local control compared to patients with lower levels of expression.

Chapter 5

In chapter 5 we report on the predictive value of clinical posttreatment factors. Treatment outcome was evaluated in 82 patients 6 to 8 weeks after treatment by magnetic resonance imaging (MRI) and examination under general anesthesia (EGA). MRI findings were compared with pre-treatment MRI data and graded for risk of local recurrence/residual disease. Out of 62 patients with masses smaller than 10 mm on post-treatment MRI, only one patient had residual disease detected by EGA 6-8 weeks after treatment. Residual disease was detected in five patients with masses larger than 10 mm on post-treatment MRI. Residual masses with a diameter > 10 mm on post-treatment MRI harbor an even higher risk (55%) of developing into a local recurrence. Post-treatment MRI emerged as an independent predictive factor for local control (hazard ratio, 3.0; p= .014). We conclude that EGA should be reserved for those patients with residual masses on MRI larger than 10 mm.

Chapter 6

Chapter 6 describes both the effectiveness and safety of salvage neck dissection after chemoradiation. In a series of 540 patients treated with chemoradiation, 127 patients had residual or recurrent neck metastases. A salvage neck dissection was performed in 61 patients. Specimens of 68 neck dissections from these patients were examined. Vital tumor cells were found in 26 patients. Of these, thirteen patients underwent a selective neck dissection and the others had a (modified) radical neck dissection. Nine patients developed a regional recurrence after salvage neck dissection of which 5 were located in the contralateral neck (predominantly in the N2c pretreatment neck). The 5-year regional control rate and overall survival rate for the 61 patients were 79% and 36%, respectively. In a multivariable analysis the status of the surgical margins in the neck specimen appeared to be the only significant prognostic factor for overall survival. USgFNAC had a sensitivity of 80% and specificity of 42% and was therefore deemed unreliable for decision making in patients treated with chemoradiation. Given the good regional control rate for the entire population, including those who underwent a salvage neck dissection, and taking into account the fact that only 24% of the N2-3 patients eventually

needed a salvage neck dissection, we conclude that a careful watch and wait strategy is safe and that a planned neck dissection after CCRT is overtreatment for the majority of patients.

Chapter 7

Knowledge of expected toxicity in chemoradiotherapy is of importance for treatment choices in head and neck cancer. In chapter 7 we describe the relationship between patient-, tumor- and treatment-related factors and acute/late toxicity in 125 patients. Mucositis, dysphagia and leucopenia were the most common reported toxicities. Neurological complications due to the intra-arterial infusions were rare. Associations between oral cavity, oropharyngeal carcinoma and severe acute mucositis; oral cavity carcinoma and osteoradionecrosis; higher age and xerostomia; and higher N-classification and persistent tube feeding was found. Only N-classification remained an independent predictive factor for persistent tube feeding in a multivariable analysis.

CONCLUSIONS

Chemoradiation can be regarded as the new standard of care for patients with functionally or anatomically unresectable advanced head and neck cancer[1]. After testing many different radiotherapy regimens as well as many different chemotherapy combinations, combination chemotherapy and concurrent hyperfractionated or accelerated radiotherapy may offer the best results in terms of cure[2,3]. Considering the fact that these high active regimens are accompanied by higher toxicity, it is important to identify those patients who will not benefit from this approach. Our research as presented in this thesis demonstrates that patients can be selected for treatment using tumor volume measurements and a nomogram based on prognostic variables. Our findings need to be externally validated as we could not demonstrate improved survival after intra-arterial administration of cisplatin compared with intravenous delivery in a large randomized trial. This holds true for the identification of a genetic profile favoring response to chemoradiation also. We were not able to identify a strong chemoradiation sensitivity gene profile apart from chromosomal differences in CGH profiles between CCRT resistant and sensitive patients.

Recently molecular targeted therapies have given positive results in combination with radiation[4,5] and some initial positive results with chemoradiation at the cost of higher incidence of acute toxicity[6,7]. It seems reasonable to foresee future validation of our established prognostic factors in randomized trials investigating the role of small molecules as novel targets for cancer therapy. From a genetic point of view, microarray research on the same tissue specimens as used for this thesis will hopefully lead to the identification of gene(s) involved in resistance to chemoradiation (J Pramana et al. IJROBP, 2007; in press). If radiotherapy is combined with molecular targets, micro-array analysis may be instrumental in finding mechanistic explanation of resistance to therapy.

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SAMENVATTING

Hoofdstuk 1

In dit hoofdstuk wordt de ontwikkeling van niet-chirurgische behandeling in de vorm van gecombineerde chemotherapie en radiotherapie voor patiënten met uitgebreide hoofdhals kanker beschreven. Uit eerder onderzoek is gebleken dat gecombineerde chemoradiatie betere curatieve mogelijkheden biedt dan adjuvante of neo-adjuvante behandelingsschema's. De eerste resultaten van radiotherapie ('RAD', 70 Gy) met gelijktijdige intra-arteriële toediening van hoge dosis cisplatinum (150mg/m^2 ,'PLAT') waren hoopvol met hoge initiële locoregionale complete respons percentages. Aan het einde van hoofdstuk 1 worden de doelen van het proefschrift samengevat om te zoeken naar klinische en biologische eigenschappen die bepalend zijn voor een goede respons op de ''RADPLAT'' behandeling. Locale tumor respons en overleving worden hierbij als belangrijkste uitkomstmaten gehanteerd.

Hoofdstuk 2

Allereerst wordt de voorspellende waarde van algemeen bekende klinische en radiologische prognostische factoren zoals leeftijd, geslacht, comorbiditeit, primaire tumor localisatie, tumor volume en TNM stadiering in 92 opeenvolgende patiënten onderzocht. De enkel- of dubbelzijdigheid van de intra-arteriele infusie werd hieraan als specifieke behandelingsgerelateerde factor toegevoegd. Het tumor volume van de primaire tumor en eenzijdige intra-arteriële infusie waren significant voorspellende factoren voor locale tumorrespons. Tumor volume werd als continue variabele gemeten waarbij per 1 cm^3 volume stijging de kans op complete remissie met 2,6% afneemt. Tumor volume van de primaire tumor, co-morbiditeit, laagste pathologische lymfeklierstation in de hals en gewichtsverlies voor behandeling waren significant voorspellende factoren voor overleving. In de multivariabele analyse bleek het primaire tumorvolume uiteindelijk de meest belangrijke factor (p= 0.003) voor selectie van patiënten voor chemoradiatie. Middels een nomogram, waarin de variabelen uit de multivariabele analyse zijn opgenomen kan de kans op locale controle en overleving voor een individuele patiënt berekend worden.

Hoofdstuk 3

Hoofdstuk 3 beschrijft de resultaten van de globale analyse van het genoom van 40 RADPLAT-patiënten met behulp van comparatieve genomische hybridisatie (CGH), waarbij het DNA van 20 chemoradiatie-sensitieve en 20 chemoradiatie-resistente patiënten werd vergeleken. Resistente tumors werden gekenmerkt door een bepaald genetisch profiel en hoog amplificatieniveau. Toename van 3q11-q13, 3q21-q26.1 en 6q22-q27 en verlies van 3p11-pter and 4p11-pter waren significant geassocieerd met resistentie tegen chemoradiatie. Unieke chemoradiatie resistentie amplificaties werden geidentificeerd op de volgende chromosomale regio's: 1p32, 3q24, 7p11.1, 7p11.2-12, 8p11.1, 8p11.1-12, 12q15, 13q21, 15q12, 18p11.3 and 18q11. Alhoewel duidelijke genetische verschillen werden vastgesteld tussen sensitieve

en resistente tumoren, blijft identificatie van kandidaatgenen, die voor deze veranderingen kunnen zorgen, noodzakelijk.

Hoofdstuk 4

Dit hoofdstuk concentreert zich op onderzoek naar kandidaatgenen die bepalend zijn voor resistentie tegen chemoradiatie. Op 92 voor behandeling afgenomen biopten, werden achttien potentieel voorspellende moleculaire markers getest met behulp van tissue micro-arrays (TMA). Met speciale aandacht werd gekeken naar de eventuele voorspellende waarde van de combinatie van een markerset. Er werden echter geen voorspellende combinatieprofielen gevonden. MRP2 en p16 bleken de enige onafhankelijk voorspellende factoren voor locale controle. HIF-1α was nagenoeg significant (p= 0.053) voorspellend voor overleving. Hoewel na univariabele analyse een correlatie werd gevonden tussen de combinatie van p16, p21, and p27 en overleving (p=0.05) bleef dit niet behouden na multivariabele analyse. Alleen MRP2 en P16 bleken daarin onafhankelijke voorspellende markers voor locale controle te zijn.

Hoofdstuk 5

Na het onderzoeken van prognostische factoren vóór behandeling, werden ook factoren onderzocht die na behandeling van belang kunnen zijn. Het resultaat van de RADPLAT behandeling werd 6 tot 8 weken na behandeling geëvalueerd door middel van onderzoek in algehele narcose en MRI beeldvorming, die met de MRI vóór behandeling vergeleken werd. Van 62 patiënten met een kleine afwijking (diameter <10mm) op de MRI had slechts 1 patiënt een aantoonbaar tumor recidief tijdens het daaropvolgend onderzoek in narcose. Bij vijf van de 20 patiënten met grotere MRI afwijkingen (diameter > 10mm) werd een recidief bevestigd tijdens het onderzoek in narcose. Radiologisch aantoonbare residuen (diameter > 10 mm) op de MRI na behandeling waren risicovoller voor het ontwikkelen van een recidief dan radiologische residuen <10mm; respectievelijk 55% en 19%. De MRI na behandeling bleek een onafhankelijk voorspellende factor voor locale controle (Hazard ratio, 3.0; p= 0.014). Het onderzoek onder narcose in combinatie met MRI na behandeling had nauwelijks meerwaarde boven MRI alleen.

Hoofdstuk 6

Hoofdstuk 6 beschrijft de effectiviteit van een halsklierdissectie na chemoradiatie in een groep van 61 patienten. Bij 26 patiënten (43%) werd een regionair tumorrecidief aangetoond waarvoor een aanvullende halsklierdissectie werd uitgevoerd. Dertien patiënten ondergingen een selectieve halsklierdissectie en 13 een gemodificeerde radicale halsklierdissectie. Negen patiënten ontwikkelden daarna een regionaal recidief, waarvan vijf in de contralaterale hals (N2c-stadiering voor behandeling, n=3). De strategie van aanvullende halsklierdissectie na RADPLAT resulteerde in een regionale controle van 79% en een overleving na 5 jaar van 36%. Irradicale resectie van de halskliermetastase vormde de enige voorspellende factor voor overleving (p< 0.001). De echografisch geleide cytologische punctie na chemoradiatie

had een sensitiviteit van 80% en een specificiteit van 42% en heeft daardoor een beperkte diagnostische waarde voor met chemoradiatie behandelde patiënten. Gelet op het feit dat aanvullende halsklierdissectie leidt tot een goede regionale controle en dat met reeds voor behandeling geplande halsklierdissecties veel onnodige halsklierdissecties worden uitgevoerd, lijkt een expectatief beleid na chemoradiotherapeutische behandeling verdedigbaar.

Hoofdstuk 7

Informatie over de te verwachten door chemoradiatie geïnduceerde toxiciteit is belangrijk voor het maken van keuzes in behandeling van patiënten met een hoofd-hals carcinoom. In hoofdstuk 7 worden de acute en late toxische effecten beschreven van 125 RADPLAT patiënten in relatie met patiënt-, tumor- en behandelingsgerelateerde factoren. Mucositis, dysphagie en leukopenie bleken de meest voorkomende toxiciteiten te zijn. Neurologische complicaties ten gevolge van de intra-arteriële infusies kwamen nauwelijks voor. Patienten met tumoren in de mondholte en orofarynx hadden een hoog risico voor het optreden van mucositis, mondholte tumoren met name voor osteoradionecrose, hogere leeftijd voor xerostomie en hogere N-stadiëring voor persisterende sondevoeding.

CONCLUSIES

Samengevat worden er in dit proefschrift een aantal significant voorspellende factoren beschreven voor de uitkomst na intra-arteriële chemoradiatie (RADPLAT) bij patiënten met uitgebreide hoofdhals tumoren. Berekening van kans op locale controle en overleving bleek mogelijk met behulp van een nomogram, waarin de significant voorspellende factoren zijn verwerkt. Omdat chemoradiatie wereldwijd in toenemende mate geaccepteerd wordt als standaardbehandeling voor patiënten met (functioneel) irresectabele hoofdhalscarcinomen en toxiciteit van deze gecombineerde behandeling hoog is, wordt het belang van kennis over prognostische factoren voor de uitkomst van behandeling ook steeds belangrijker. We konden echter geen voordeel van intra-arteriële chemoradiatie boven intraveneuze chemoradiatie vinden in een onlangs gesloten fase III studie. Daarom zullen de voorspellende factoren uit dit proefschrift extern gevalideerd moeten worden.

Aangezien recente studies waarin moleculaire therapie (zg. 'small molecules') in combinatie met radiotherapie werd onderzocht een positief resultaat gaven in patiënten met uitgebreide hoofdhals kanker, lijkt het logisch om toekomstige validatie van onze voorspellende factoren te onderzoeken in gerandomiseerde studies die moleculaire behandeling als nieuwe therapie onderzoeken. Vanuit een genetisch perspectief zullen nieuwe resultaten van een al gestart micro-array onderzoek hopelijk leiden tot meer inzicht in genen die een rol spelen in chemoradiatie resistentie.

Dankwoord

DANKWOORD

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