ARCON FOR LARYNGEAL CANCER

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General introduction and outline of the thesis

Geert O. Janssens

General introduction and outline of the thesis

Laryngeal carcinoma

The estimated number of new patients presenting with laryngeal cancer in the Netherlands is around 700 per year [1]. The disease is more prevalent in males than females with a factor 7:1. While the incidence in men is decreasing, a stabilization is observed in women. A clear association has been made with smoking, excess alcohol ingestion, and more recently human papilloma virus [2,3].

The larynx is divided into the following three **anatomical regions**:

- The *supraglottic larynx* includes the epiglottis, false vocal cords, ventricles, aryepiglottic folds, and arytenoids.
- The *glottis* includes the true vocal cords and the anterior and posterior commissures.
- The *subglottic region* begins 1 cm below the true vocal cords and extends, depending on definitions, to the lower border of the cricoid cartilage or the first tracheal ring.

The supraglottic area is rich in **lymphatic drainage**. After penetrating the pre-epiglottic space and thyrohyoid membrane, lymphatic drainage is initially to the jugulodigastric and midjugular nodes. The true vocal cords are devoid of lymphatics. Extension above or below the cords may, however, lead to lymph node involvement. Primary subglottic cancers drain through the cricothyroid and cricotracheal membranes to the pretracheal, paratracheal, and inferior jugular nodes, and occasionally to mediastinal nodes.

Squamous cell carcinoma is by far the most prevalent histopathological diagnosis. Two-thirds of the laryngeal cancers are located at glottic level, one third at supraglottic level and the subglottic localization is very rare [1]. Men have more glottic tumors (64%), and women more supraglottic tumors (54%) [1]. Because of persistent hoarseness, the majority of glottic tumors is diagnosed at an early stage (stage I: 60%; stage II: 29%) [1]. Atypical symptoms at presentation can explain the more advanced stage (stage III: 23%; stage IV: 44%) of supraglottic tumors at diagnosis [1].

Optimal **functional larynx preservation** is the main goal when treating patients with laryngeal cancer. For early stages, treatment comprises (laser) surgery or radiotherapy with excellent long-term results [4]. Since the landmark trial, conducted by the Department of Veterans Affairs Laryngeal Cancer Study Group, demonstrated that induction chemotherapy followed by definitive radiotherapy did not compromise survival when compared to initial laryngectomy, organ-sparing approaches became

preferred treatment for the majority of patients with advanced laryngeal cancer [5]. In the subsequent randomized Radiation Therapy Oncology Group 91-11 trial, the 5 year local- and loco-regional control rate in patients treated with radiotherapy and concurrent cisplatin (71% and 68%) differed significantly from that for patients given induction chemotherapy followed by radiotherapy (58% and 55%) or conventional fractionated radiotherapy alone (54% and 51%) [6]. This means that still a significant subset of patients with advanced laryngeal cancer will develop loco-regional disease recurrence. **Tumor-cell repopulation** and **hypoxia (the lack of oxygen)** are important mechanisms of resistance that can cause therapy failure.

Tumor-cell repopulation

In squamous-cell carcinomas, the probability of local tumor control decreases as the overall treatment time of radiotherapy increases without a change in the radiation dose [7,8]. This relation is explained by an increase in the net production of clonogenic tumor cells during radiotherapy. Although during a course of radiotherapy the number of tumor cells with clonogenic potential is greatly reduced, cells that survive are triggered to repopulate more effectively [8]. To counteract the clonogenic repopulation during therapy, overall treatment time is reduced by giving the same total radiation dose with several fractions per day. This strategy is called accelerated fractionated radiotherapy. However, there is a limit to this acceleration because rapidly renewing normal tissues, such as mucosa, also rely on repopulation for the repair of tissue integrity after radiation damage [9]. There is now evidence from a Cochrane Systematic Review that accelerated radiotherapy without total dose reduction for patients with head-and-neck cancer, improved loco-regional tumor control at 5 years by 7.3% compared to conventional radiotherapy [10]. Successful studies generally used schedules with a reduced total treatment time of 5.0 to 5.5 weeks and an unchanged total dose of around 70 Gy.

Hypoxia

Tumor-cell hypoxia is another well-recognized cause of resistance to radiation. Oxygen mediates the biological effects of ionizing radiation, and the response of cells to radiation depends strongly on the availability of oxygen. Typically, hypoxic cells are 2.5-3.0 times more radioresistant than well-oxygenated cells [11]. In the majority of solid tumors there is some imbalance between oxygen delivery and oxygen consumption, resulting in hypoxia [12]. The transport of oxygen to cells relies on blood perfusion and diffusion through the tissues. However, a chaotic vascular system impedes delivery of oxygen to the respiring neoplastic cells in solid tumors [13]. The consequences of these abnormalities are impaired blood perfusion and increased diffusion distances leading to a large temporal and spatial variability in tumor oxygenation.

Hypoxia also *promotes more aggressive tumor behavior.* Hypoxia is a powerful trigger for changes in gene expression and associated changes in the micro-environment. Due to these changes, clonogenic cells with increased adaptation to hypoxia are stimulated, driving the tumor towards a more malignant phenotype [14].

ARCON

ARCON, acronym for accelerated radiotherapy with carbogen and nicotinamide, has been developed to counteract tumor-cell repopulation and hypoxia, both mechanisms of radiotherapy resistance. To limit clonogenic repopulation during therapy, the overall duration of radiotherapy is reduced from 7 weeks to 5.5 weeks, by delivering two fractions per day during part of the treatment. This accelerated radiotherapy is combined with inhalation of a hyperoxic gas (carbogen: 95-98% oxygen + 2-5% carbon dioxide) to decrease diffusion-limited hypoxia, and nicotinamide, a vasoactive agent, to decrease perfusion-limited hypoxia (**Figure**).

Preclinical studies have been done to test the enhancing effects of the three components of ARCON in experimental mouse tumors and normal tissues. Phase 1 and 2 clinical trials have shown feasibility, tolerability and promising results of ARCON. More recently, a phase III trial for larynx carcinoma was completed which is subject of this thesis.

Carbogen

Raising arterial partial pressure of oxygen (pO_2) by inhalation of hyperoxic gases is the most effective way to decrease diffusion-limited hypoxia. A small fraction of carbon dioxide was added to oxygen to increase the respiratory drive, to induce vasodilatation

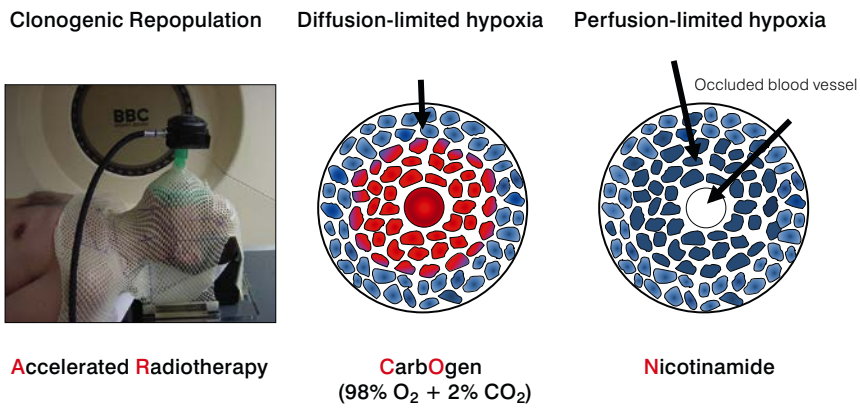

Figure The concept of ARCON therapy.

but also for the improved oxygen delivery from blood to the tissues by a right-shift of the hemoglobin-oxygen dissociation curve. However, studies investigating the radiosensitizing effect of the addition of carbon dioxide to oxygen remain controversial [15,16]. The increase of the oxygen diffusion distance with inhalation of carbogen is best demonstrated by immuno-histochemical studies [17]. Irreversible intracellular binding occurs with nitroimidazoles, like pimonidazole, at tissue pO_2 below 10 mmHg. The areas with hypoxic-marker binding become smaller with inhalation of carbogen and distances from vessels to hypoxia increase. Animal and human studies showed that a carbogen prebreathing time of 5 minutes was required before optimum tumor oxygenation occurred and the maximum sensitizing effect was achieved [15;18; 19].

Nicotinamide

Nicotinamide is the amide derivate of vitamin B_3 and has been studied for its radiosensitizing properties [20;21]. It is likely to act by decreasing perfusion-limited tumor hypoxia through prevention of intermittent vascular shutdown. Additional effects on tumor metabolism are thought to be possible as well [22]. Nicotinamide is converted to nicotinamide adenine dinucleotide (NAD^+). Increased concentrations of NAD^+ would lead to stimulation of many metabolic processes, including oxidative metabolism and glycolysis, with shifts in the oxygen consumption rate and acid-base balance. These effects could then have secondary effects on tumor blood flow and tissue pO_2.

For the radiosensitizing effect, a plasma concentration of 700 μmol/L (equivalent to an oral dose of 80 mg/kg) is required. However, daily administration of this dose over a course of 5-7 weeks caused nausea and vomiting with discontinuation of intake in a large proportion of patients [23]. A reduction in peristalsis due to interaction with the gastro-intestinal smooth muscle, was the reason for the gastro-intestinal side-effects [24]. Compliance significantly improved with a reduction in dose to 60 mg/kg, given 1-2 hours before radiotherapy and domperidone as anti-emetic profylaxis [25].

Preclinical studies

In mouse mammary tumors, Rojas et al. observed an enhancement ratio of 1.19 for accelerated radiotherapy compared to conventional radiotherapy [26]. When carbogen was added to the accelerated regimen, a further enhancement was seen (ratio: 1.71). Finally, the combination of accelerated radiotherapy, carbogen, and nicotinamide resulted in an enhancement ratio of 1.91, indicating that with ARCON the same effect could be achieved as with conventional radiotherapy but with a radiation dose almost 50% lower.

While enhancement ratios of 1.0-2.4 are observed for murine tumors, enhancement ratios of the normal tissues are much lower (ratio 1.0-1.2) [27-31]. This indicates that, potentially, a significant therapeutic gain can be obtained with the ARCON approach.

Clinical studies

In the early 1990s, the first feasibility and toxicity studies with ARCON were done at Mount Vernon Hospital in the UK, shortly followed by other centers and the European Organisation for Research and Treatment of Cancer (EORTC) [32,33]. The initial phase I and II studies introduced ARCON in consecutive stages to analyze the feasibility and toxic effects of each component. The largest experience with ARCON is in patients with head and neck cancer, a disease with well-documented existence of hypoxia and tumor-cell repopulation [8;34;35]. In general, there was an increase in early mucosal and skin reactions observed with accelerated radiotherapy, mainly manifested as a delayed recovery with longer healing times [36]. Although the absolute number was small, an increased rate of patients with mandibular necrosis was observed [37;38]. Only the study of Kaanders et al. was large enough to allow conclusions about tumor response [37]. A group of 215 patients with advanced tumors (mainly T3 or T4) of the larynx, hypopharynx, oropharynx, and oral cavity were treated with accelerated radiotherapy (64-68 Gy in fractions of 2 Gy over 36-38 days, 2 fractions per day during the last 1.5 weeks) combined with carbogen and nicotinamide. Local control rates were 80% for laryngeal, 60% for hypopharyngeal, 87% for oropharyngeal, and 29% for oral-cavity tumors. The high local control rate for advanced laryngeal cancers, approaching the results reported for T1 and T2 lesions, supported the concept of increased susceptibility of tumors to the biologically based approach of ARCON, offering excellent possibilities for organ preservation. Interestingly, T-stage had no prognostic significance, assuming that biological factors are more important predictors of treatment outcome.

Patient selection for ARCON

Between tumors of the same site and histology, large heterogeneity in biological characteristics exists. Identification of proliferative activity and oxygenation status in tumors is a logic approach to select candidates for ARCON.

In head and neck cancer a high proliferation index is correlated with aggressive tumor characteristics and poor outcome in numerous studies [39]. There are several indicators of the proliferation rate of tumors like growth fraction, a specific phase of the cell cycle or the cell cycle time. A widely used endogenous immunohistochemical marker is Ki-67. Recently, it was demonstrated in a large cohort of patients with advanced laryngeal cancer treated in the phase III ARCON study that node positive patients had a higher Ki-67 labeling index compared to node negative patients. In the

group of patients treated with AR alone, high Ki-67 labeling index was associated with increased regional and distant metastasis formation whereas this was not observed in patients treated with ARCON [40]. The fact that accelerated radiotherapy does not correct for the negative prognostic value of Ki-67 while the addition of hypoxic modification does, does suggest a mutual relationship between proliferation and hypoxia.

The gold standard method to measure pO_2 is the use of Eppendorf polarographic electrodes [41]. However, the invasive and technically demanding nature of the technique is not attractive for routinely use. For this reason, exo- and endogenous hypoxia marker assays, detectable by immunohistochemistry, and more recently PET-tracers depicting hypoxia are more relevant in clinical use. The exogenous marker Pimonidazol can be administered intravenously and binds to viable hypoxic cells. In head and neck cancer a correlation was found between the degree of hypoxia, estimated by pimonidazol binding, and the loco-regional control and event-free survival [42;43]. In a group of 38 patients with head and neck cancers from different subsites, Kaanders et al. demonstrated that loco-regional control was significantly lower for patients with high pimonidazol binding levels (hypoxic tumors). However, this association disappeared in the subgroup of patients treated with ARCON [42]. A method not relying on injected markers to quantify hypoxia is the use of endogenous markers. Endogenous markers, like hypoxia-inducible factor (HIF), carbonic anhydrase IX (CA-IX) or glucose transporters (GLUTs) are proteins upregulated under hypoxic conditions. No single endogenous marker for head and neck cancer has consistently demonstrated strong prognostic power in clinical practice [44-46]. Attempts to combine various markers to create a hypoxia-prognostic profile are with moderate success [47,48]. In the field of PET-imaging, multiple hypoxia-related tracers have been tested during the years [49]. Among the various PET tracers synthesized, 18F-Misonidazole (MISO) and 18F-Fluoroazomycin arabinoside (FAZA) are the most commonly used. A major advantage of hypoxia imaging by PET is the geographic distribution of hypoxia within the tumor volume and its evolution during treatment. After co-registration of molecular imaging with radiotherapy CT- or MR-imaging, customized heterogeneous dose distributions can be generated with dose escalation to areas of hypoxia in order to improve loco-regional control [50;51]. Response measuring early during radiotherapy can discriminate good-responders from poor-responders and also result into treatment adaptation [52;53].

Outline of the thesis

A 2-year local control rate of 92%, observed in 62 patients with stage III-IV laryngeal cancer treated in the phase II study, prompted a multicenter phase III study [54]. From 2001 until 2008, 345 patients with cT2-4 laryngeal cancer were randomly assigned to accelerated radiotherapy or accelerated radiotherapy combined with carbogen breathing and nicotinamide. This thesis reports on outcome, toxicity profile, role of anemia, impact of tumor volume and health-related quality-of-life (HRQoL). Two-years after inclusion of the last patient, general *outcome* of the phase III trial is analyzed and reported in **Chapter 2**. A translational side-study, using the exogenous hypoxic marker pimonidazole, addresses the issue of selecting patients based on the oxygenation status.

The potential price of treatment intensification is an increase of toxicity. **Chapter 3** reports on the acute *toxicity* observed during and after radiotherapy. Skin, mucosa and mucosa-related symptoms as well as compliance are compared between AR and ARCON.

Anemia is associated with poor tumor control. Results from the phase II study demonstrated that ARCON can correct this adverse outcome in patients with head and neck cancer. In **Chapter 4**, the impact of ARCON on a subgroup of patients presenting with anemia is investigated.

Retrospective studies indicate that larger tumor volume is a strong prognostic indicator for poor tumor control after (chemo)radiotherapy for laryngeal cancer. The impact of tumor volume on the outcome of patients with locally advanced laryngeal cancer treated in the phase III ARCON trial is reported in **Chapter 5**.

Proper knowledge of HRQoL is equally essential to understand the real benefit of a new regimen. HRQoL is assessed using the European Organisation for Research and Treatment of Cancer (EORTC) HRQoL Questionnaire-C30 (QLQ-C30) and the Head & Neck cancer module (QLQ-H&N35) at baseline, at completion of radiotherapy and at 6, 12, and 24 months post-baseline. Results are discussed in **Chapter 6**.

A general discussion, based on the abovementioned chapters, is given in **Chapter 7**. **Chapters 8 and 9** provide a summary of the work in English and Dutch.

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2

Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: results of a phase III randomized trial

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Summary

Purpose

To report the results from a randomized trial comparing Accelerated Radiotherapy (AR) with Accelerated Radiotherapy plus Carbogen and Nicotinamide (ARCON) in laryngeal cancer.

Patients and Methods

Patients with cT2-4 squamous cell laryngeal cancer were randomized to AR (68 Gy within 36-38 days) or ARCON (AR plus carbogen inhalation and nicotinamide). To limit the risk of laryngeal necrosis, ARCON patients received 64 Gy on the laryngeal cartilage. The primary endpoint was local control. Secondary endpoints were regional control, larynx preservation, toxicity, disease-free survival and overall survival. In a translational side study the hypoxia marker pimonidazole was used to assess the oxygenation status in tumor biopsies.

Results

From 04-2001 to 02-2008, 345 patients were accrued. After a median follow-up of 44 months, local tumor control rate at 5 years was 78% for AR versus 79% for ARCON ($P=.80$) with corresponding larynx preservation rates of 84% and 87% ($P=.48$). The 5-year regional control was significantly better with ARCON (93%) compared to AR (86%, $P=.04$). The improvement in regional control was specifically observed in patients with hypoxic tumors and not in patients with well-oxygenated tumors (100% versus 55% respectively, $P=.01$). AR and ARCON produced equal levels of toxicity.

Conclusions

Despite lack of benefit in local tumor control for advanced laryngeal cancers, a significant gain in regional control rate, with equal levels of toxicity, was observed in favor of ARCON. The poor regional control of patients with hypoxic tumors is specifically countered by ARCON treatment.

Introduction

Functional larynx preservation is a main goal when treating patients with loco-regional advanced laryngeal cancer.[1,2] The combination of radiotherapy with concurrent cisplatin demonstrated better larynx preservation and loco-regional control compared to conventional fractionated radiotherapy alone.[2] However, conventional fractionated radiotherapy is currently not considered the optimal strategy for head and neck cancer. Based on accomplishments of radiobiological research, new approaches in clinical radiotherapy have been developed to improve treatment outcome.

In head and neck cancer, tumor cell repopulation and tumor hypoxia are known factors determining radiation response. Accelerated Radiotherapy plus Carbogen and Nicotinamide (ARCON) is a strategy to counteract these resistance mechanisms.[3] To limit clonogenic repopulation during therapy, the overall duration of radiotherapy is reduced by delivering multiple fractions per day. This approach, referred to as accelerated fractionation (AR), has demonstrated superior loco-regional control rates in head and neck cancer.[4,5,6] ARCON combines accelerated radiotherapy with the inhalation of carbogen (98% O_2 + 2% CO_2) to decrease diffusion-limited hypoxia and the administration of nicotinamide, a vasoactive agent, to decrease perfusion-limited hypoxia.[7,8,9,10,11,12] Phase I and II trials have shown the feasibility and tolerability of ARCON for head and neck cancer and have produced promising results in terms of tumor control.[13,14,15] A favorable 5-year local control rate of 80% was obtained in a series of 79 T3 and T4 larynx carcinomas.[14]

This provided the basis for a multicenter trial randomizing patients with cT2-4 laryngeal cancer between AR and ARCON, of which the results are reported here. Data from this study on acute toxicity and compliance have been published recently.[16] Additionally, in a translational side study the value of a tumor hypoxia assay to predict response to ARCON was assessed. For this purpose pimonidazole was used, a bioreductive chemical probe that forms protein adducts in viable hypoxic cells and can be visualized in tumor biopsies by immunofluorescence.[17]

Patients and methods

Study Design and Eligibility

This was an open-label, randomized phase III trial comparing AR with ARCON in laryngeal cancer. The trial was conducted under the auspices of the Dutch Head and Neck Cancer Group and the Dutch Cancer Society (KWF) in 7 centers in 2 countries (Table 1).

Table 1 Patient Demographics and Clinical Characteristics.

Characteristics	AR N=174 No.	%	ARCON N=171 No.	%
Age (years)				
Median	60		61	
Range	38-88		41-84	
Sex				
male	136	78	142	83
female	38	22	29	17
Performance status				
0	140	80	137	81
1	34	20	34	19
Site of the primary tumor (%)				
Supraglottic	100	57	97	56
Glottic	74	43	74	44
T-stage (%)				
T2	67	38	55	32
T3	80	46	95	56
T4	27	16	21	12
N-stage (%)				
N0	117	67	116	68
N1	20	12	23	13
N2a	4	2	7	4
N2b	10	6	5	3
N2c	23	13	20	12
N3	0	0	0	0
Participating Institutions				
Radboud University Nijmegen Medical Centre, Nijmegen	75	43	76	44
University Medical Center Utrecht, Utrecht	38	22	39	23
VU University Medical Center, Amsterdam	21	12	20	12
University Medical Center Groningen, Groningen	15	9	13	8
Maastricht University Medical Centre, Maastricht	15	9	13	8
Leiden University Medical Center, Leiden	8	4	9	5
Mount Vernon Hospital, Northwood, UK	2	1	1	0

Eligibility was assessed by a multidisciplinary head and neck oncology team. Diagnostic workup consisted of full history and physical examination, blood cell count and blood biochemistry, laryngoscopy under general anesthesia with biopsy taking, CT- or MR-imaging of the larynx and neck, ultrasound-guided fine needle aspiration of suspect lymph nodes and chest X-ray. All patients over the age of 18, WHO performance status ≤1 and squamous cell carcinoma of the larynx and the following clinical stage (TNM-classification, UICC 1997), were considered for this study: T2 glottic carcinoma with impaired cord mobility or subglottic extension, T2 supraglottic carcinoma with invasion of the mucosa of the base of tongue or vallecula or invasion of the medial wall of the piriform sinus, T3-4 glottic or supraglottic carcinoma and any N-stage but M0.

Exclusion criteria included prior or concurrent treatment for this tumor, severe stridor with impossibility for adequate debulking of airway, impaired renal and/or hepatic function (creatinine > upper normal limit, ASAT/ ALAT >1.5 times upper limit), use of nefrotoxic or anti-convulsant medication that could not be discontinued and a history of malignancy during the previous 5 years (with exception of basal cell carcinoma of the skin, carcinoma in situ of the cervix or superficial bladder cancer).

Approval for the study was obtained from the Radboud University Nijmegen Medical Centre research Ethics Committee with ratification from each centre before start. Written informed consent and completed quality of life questionnaire were obtained before randomization.

Sample Size

The target sample size was 344 patients (n= 172 per arm), determined to provide 80% power to detect a difference of 60 vs. 75% in local control rate at 2 years for AR vs. ARCON, respectively, allowing for dropouts and a significance level of .05 (two-sided log-rank test). Follow-up of 2 years after the last inclusion, was respected before data analysis.

Randomization and masking

Patients fulfilling enrollment criteria were centrally randomized by phone at the IKO (Integraal Kankercentrum Oost) trials office. Treatment arm assignments (AR vs. ARCON) were stratified for tumor site (glottic vs. supraglottic) and institution. A dynamic allocation method was used to avoid imbalance of treatment assignment within an institution. Randomization took place after all study investigations and no longer than 4 weeks prior to the anticipated start of treatment. Data were unmasked.

Treatment

A CT-scan in treatment position with immobilization device was used in all the patients. The initial radiation planning target volume encompassed the primary tumor and the lymph node levels II, III and IV in case of cN0. Level V and VI were included in case of cN1-3 and >1.0 cm subglottic extension, respectively.[18] The boost planning target volume encompassed the primary tumor volume and the macroscopic involved lymph nodes. A total dose of 44 Gy in 22 daily fractions of 2 Gy was prescribed followed by a boost dose of 24 Gy in twice daily fractions of 2 Gy with a minimum interval of 6 hours between the fractions. Dose specification and dose homogeneity requirements were according to Report 50 of the ICRU.[19] The overall treatment time was 36-38 days. Because a decrease in radiation tolerance was observed for cartilage and spinal cord in earlier studies with hypoxic sensitization, the total dose to the arytenoid cartilage and the spinal cord in the ARCON arm was limited to 64 and 40 Gy, respectively.[20,21]

Patients allocated to the ARCON arm received carbogen (98% O_2 + 2% CO_2, 4 minutes before and during daily fractions) and oral nicotinamide (60 mg/kg, 1-1.5h before each fraction) concurrently with radiotherapy.[22,23] During the boost nicotinamide was given only before the first fraction of the day. To prevent nausea, domperidone (10 mg, thrice-daily dosage) was given.

Hypoxia marker side study

After informed consent, patients received pimonidazole (Hypoxyprobe-1; Natural Pharmacia International, Belmont, MA) intravenously (500 mg/m^2) two hours before biopsy taking. Biopsies were snap frozen in liquid nitrogen, immunohistochemically stained and semi-automatically analyzed as described earlier.[24] Of each biopsy, one complete section was analyzed to define the hypoxic fraction, i.e. the area positive for pimonidazole relative to the total tumor area. Based on previous pimonidazole marker studies, a cut-off value of 2.6% was used to dichotomize between well-oxygenized and hypoxic tumors.

Monitoring During Treatment and Follow-up Evaluations

Acute radiation toxicities were graded according to an earlier validated list of toxicity criteria (online only table 1).[13] Any other side effects felt to be related to carbogen or nicotinamide and the reason for interruption or discontinuation were recorded as well. After resolution of the acute radiation toxicities, follow-up visits took place every 2, 3, 4 months during the first, second and third year respectively, then every 6 months for another 2 years. The larynx was assessed by fiberoptic or indirect laryngoscopy. Regional control was assessed by palpation of the neck. On suspicion of nodal recurrence an ultrasound with fine needle aspiration cytology was performed.

Recurrences were cytologically or pathologically confirmed and documented by CT-scan or MRI. Eligibility for salvage neck dissection was assessed by a multidisciplinary head and neck oncology team. Severe adverse events were defined as either events that are fatal, life-threatening or resulting in permanent disability or any late toxicity like deep mucosal necrosis, cartilage necrosis or osteoradionecrosis requiring surgery.

Endpoints and Statistics

The primary endpoint of the study was local tumor control. Local control was taken as freedom of first recurrence at the primary tumor site. Secondary endpoints were regional control, larynx preservation, toxicity, quality of life, disease-free survival (DFS) and overall survival (OS). Regional control was defined as freedom of first regional recurrence. Disease-free survival was defined as the time to a local or regional recurrence, distant metastasis, or death from any cause. Overall survival was defined as time to death. For larynx preservation, treatment was considered to have failed on the date laryngectomy was performed.

Statistical analyses were performed using SPSS 16.0.1. Survival estimates were obtained using the Kaplan-Meier method and all analyses were based on intent-to-treat policy. All intervals were calculated from the date of randomization and censored after 60 months or at last follow-up. Differences were compared using the log-rank test and hazard ratios (HR) and their 95% confidence interval (CI) were obtained using the Cox proportional hazards model. Differences in prevalence of worst grade acute and late toxicities between both treatment arms were compared using the Chi-square test.

Results

Patient Characteristics and Protocol Compliance

Between April 2001 and February 2008, 345 patients were randomized to either AR or ARCON (Figure 1). The median follow-up time was 44 (range 18-103), 55 and 60 months for the whole group and for patients still alive receiving AR and ARCON, respectively. Patient demographics and clinical tumor characteristics were well balanced without significant differences between the groups (Table 1). Compliance to radiotherapy, carbogen breathing and nicotinamide intake was high.[16] Radiotherapy was delivered as planned to 173 (99%) AR and 169 (99%) ARCON patients and was completed within the specified time of 38 days for 168 (97%) AR and 163 (96%) ARCON patients. Full compliance to carbogen breathing, nicotinamide intake and the combined treatment (ARCON) was 86%, 80% and 76%, respectively.

Tumor Control and Survival

Five-year larynx preservation rates were 84% and 87% for AR and for ARCON (P=.48). There was no significant difference in local control rate: 80% and 78% for AR versus 83% and 79% for ARCON at 2 and 5 years, respectively (HR .94; CI .58–1.52; P=.80; figure 2A). The 2- and 5-year regional control rates were significantly better with ARCON (88% and 86% for AR versus 95% and 93% for ARCON, respectively (HR .46; CI .22-.97; P=.04; figure 2B). No significant differences were found for DFS (HR .75, CI .50–1.13, P=.16; figure 2C) and OS (HR 1.03; CI .73–1.46; P=.86; figure 2D). In exploratory subgroup analysis we did not find significant differences in 5-year local control rate between treatment arms when stratified for T-stage or laryngeal subsite. Salvage laryngectomy was attempted in 29 of 35 AR and 21 of 28 ARCON recurrences. Salvage neck dissection was attempted in 12 of 21 and 8 of 12 patients treated by AR and ARCON, respectively. The ultimate 5-year local and regional control rates, including salvage therapy were 94% vs. 92% (P=.60) and 92% vs. 98% (P=.21) for AR and ARCON, respectively.

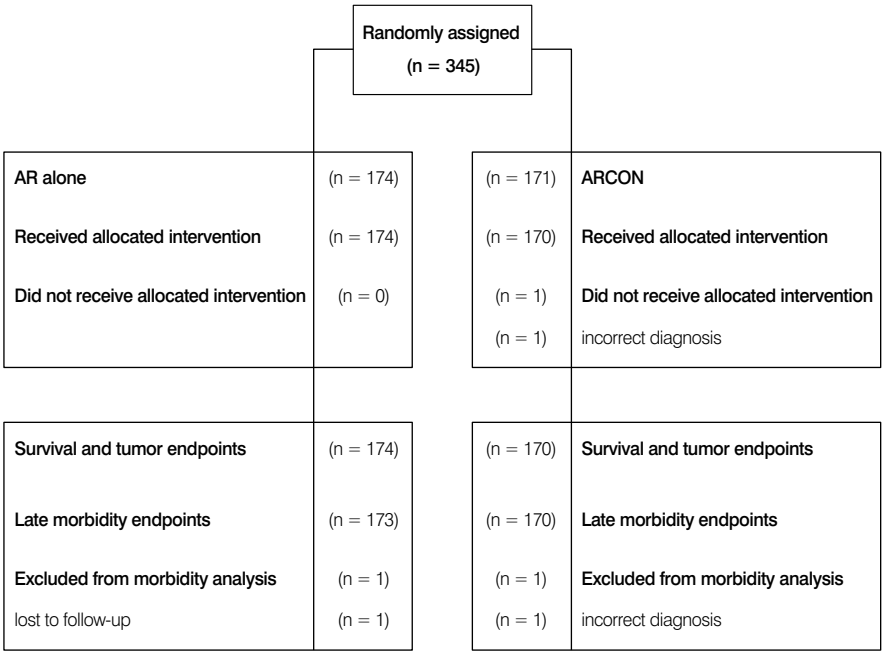

Figure 1 CONSORT flowchart.

AR, Accelerated Radiotherapy; ARCON, Accelerated Radiotherapy with Carbogen and Nicotinamide

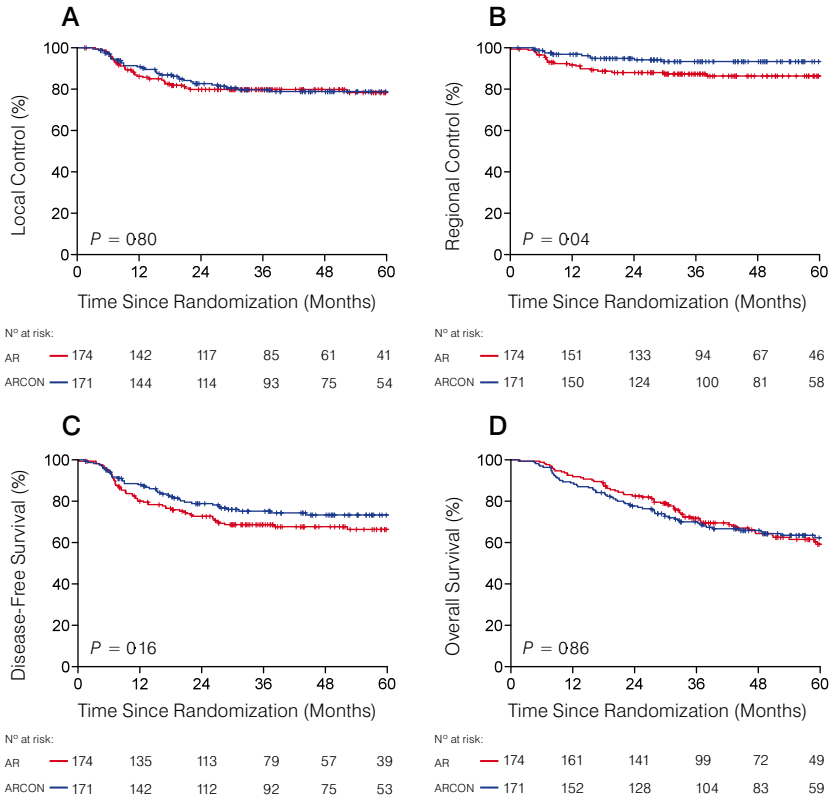

Figure 2 Kaplan-Meier curves for local control (**A**), regional control (**B**), disease-free survival (**C**) and overall survival (**D**) comparing accelerated radiotherapy only (AR) or accelerated radiotherapy plus carbogen and nicotinamide (ARCON). Log-rank P values are shown and number of patients at risk against yearly intervals.

Translational side study

Two centers participated in the hypoxia marker study and included 79 patients. The hypoxic fraction, as defined by pimonidazole staining, varied from 0-19.4% with a median value of 1.5%. There was no ascertainable benefit from ARCON with regard to local control, neither in the well-oxgenated tumors, nor in the hypoxic tumors (Figure 3A,B). However, regional control in the group with a high hypoxic fraction was significantly improved with ARCON compared to AR (100% vs. 55%, *P*=.01), while no difference between the treatment arms was observed in the group with a low hypoxic fraction (96% for ARCON vs. 92% for AR, *P*=.70) (Figure 3C,D). Patients with hypoxic

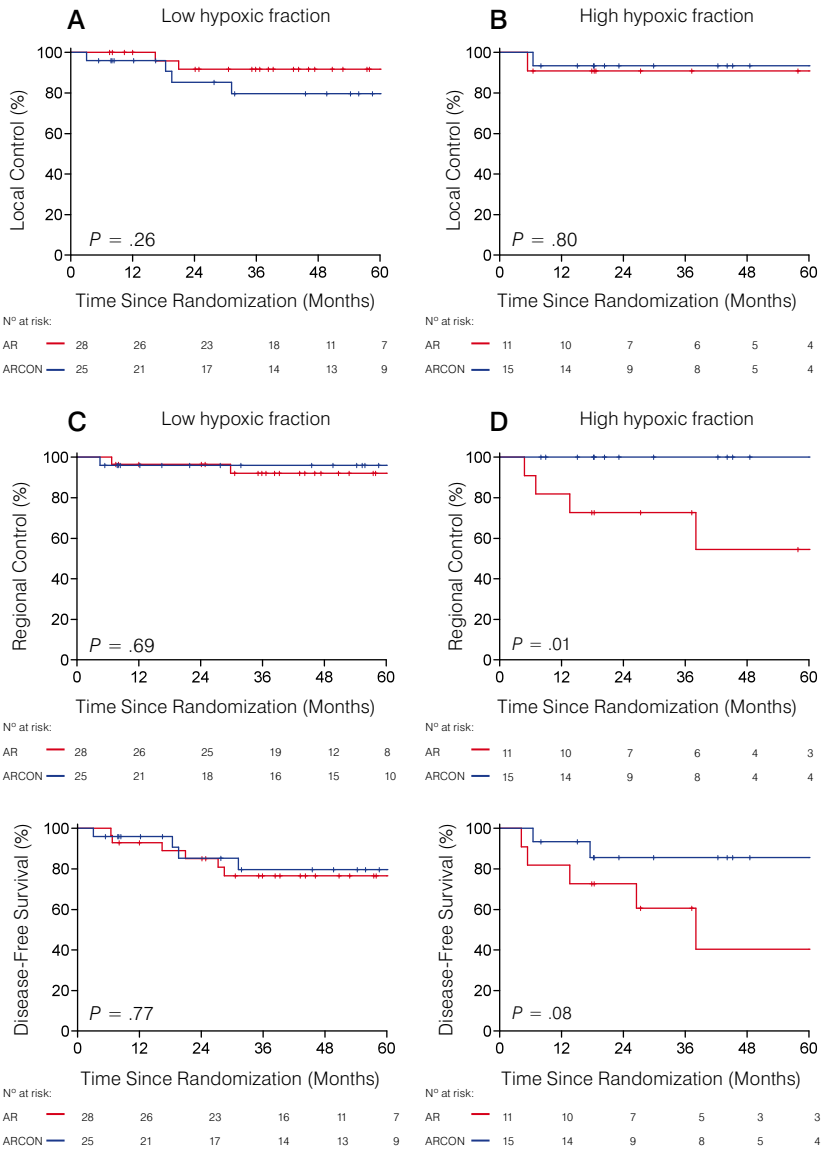

Figure 3 Local control (**A**,**B**), regional control (**C**,**D**) and disease-free survival (**E**,**F**) by oxygenation status of the primary tumor and treatment arm. Log-rank P values are shown and number of patients at risk against yearly intervals.

tumors had a substantially higher five-year DFS in the ARCON arm (86%) compared to AR (40%), although the difference did not reach the significance level (*P*=.08) (Figure 3E,F). No DFS benefit from ARCON was observed in patients with well oxygenated tumors (80% for ARCON vs. 77% for AR, *P*=.80).

Toxicity

Toxicity data have been described extensively in a recent publication.[16] Between both treatment arms (AR vs. ARCON) no statistically significant difference was observed for incidence of acute skin reactions (moist desquamation: 56% vs. 58%, *P*=.80), acute mucosal reactions (confluent mucositis: 79% vs. 85%, *P*=.14) and symptoms related to acute mucositis (severe pain on swallowing: 53% vs. 58%, *P*=.37; nasogastric tube feeding: 28% vs. 28%, *P*=.98, narcotic medicines required: 58% vs. 58%, *P*=.97). There was a small but statistically significant difference in median duration of confluent mucositis in favor of AR (2.0 vs. 3.0 weeks, *P*=.01).

Analysis of late radiation morbidity did not reveal significant differences between AR and ARCON for skin and subcutaneous tissues (severe teleangiectasia: 8% vs. 8%, *P*=.56; severe subcutaneous fibrosis: 7% vs. 9%, *P*=.26; severe subcutaneous edema, 8% vs. 4%, *P*=.09) and mucous membranes (mucosal ulceration, 8% vs. 6%, *P*=.36; nasogastric tube feeding, 6% vs. 6%, *P*=.61), respectively. Twelve patients in the AR group and 6 patients in the ARCON group received a tracheostomy for severe edema with dyspnea and/or stridor. All patients who developed a cartilage necrosis could be managed conservatively. Four of them did receive a tracheostomy (n=1 for AR, n=3 for ARCON) and there was no need for laryngectomy. One year after diagnosis 9% of the patients with the larynx in situ assigned to AR and 7% of patients in the ARCON group could swallow only soft foods or liquids and 3% of patients in both groups needed nasogastric tube feeding (*P*=.88).

Severe adverse events

One patient (AR) died from a cardiac arrest during treatment. The cause of death was unrelated to treatment. Another patient (ARCON) died one day after the end of treatment due to a gastric bleeding. He was using diclofenac for abdominal pain. Causality with nicotinamide was considered uncertain for this patient. Upper airway obstruction during radiotherapy, requiring tracheostomy, occurred in 4 patients treated by AR and in 1 patient with ARCON. Furthermore, in the ARCON arm one patient developed a renal insufficiency and sepsis and one patient was admitted to the hospital with severe bleeding from the tumor.

Discussion

The addition of carbogen and nicotinamide to a schedule of accelerated radiotherapy produces a significant gain in regional control relative to accelerated radiotherapy alone but no benefit in local control in patients with T2-T4 laryngeal cancer. This improvement in regional control can be entirely attributed to the patients with hypoxic tumors as assessed by pimonidazole staining.

Although there was no improvement in local control with ARCON, the result in the experimental arm was consistent with the 80% local control rate at 5 years obtained in the preceding phase II ARCON trial.[14] Unexpectedly, the local control rate in the control arm was higher than the 63-76% and 43-50% local control rates observed for T3 and T4 laryngeal cancers treated by hyperfractionated and accelerated regimens reported at the time of onset of this study.[25,26] A possible explanation might be stage migration due to better diagnostic imaging, especially when comparing the results of a prospective study with retrospective reports of 15 years ago. In the current study no significant differences between T-stages were observed. With conventional fractionation schedules local control rates are in the order of 67-80%, 30-77% and 26-52% for T2, T3 and T4 tumors, respectively, indicating that patients with more advanced local disease profit most from accelerated fractionation.[27,28,29,30,31]

Because the purpose of this study was to arrive at improved tumor control with no increase of late laryngeal toxicity, a dose reduction to 64 Gy for the larynx was prescribed for patients in the ARCON arm. This dose reduction was based on a decrease in radiation tolerance in the order of 10% observed for cartilage when radiotherapy was given in hyperbaric oxygen.[20] In the current study a similar effect was expected from normobaric carbogen with nicotinamide. This 4 Gy absolute dose difference on the larynx is a possible explanation for the lack of additional benefit in local tumor control with ARCON as compared to AR alone. Based on clinical data, dose-response curves have been constructed for head and neck carcinomas.[32] From these, dose-response gradients (gamma value) have been derived as a measure of the steepness of the curve. Typical gamma values for larynx carcinoma range from 1.5-2.5. This means that for each percent increment in dose, the probability of controlling the tumor will increase by approximately 2 percentage points. Given the same local control but with 4 Gy less dose, an enhancement of tumor control probability of 10-15% can be derived when carbogen and nicotinamide are combined with radiotherapy. Thus, although a dose difference of 4 Gy seems modest, the corresponding difference in cure rate is significant.

In contrast to the primary tumor, the dose delivered to the lymph node metastases in the current study was similar (68 Gy) in both arms. The addition of carbogen and nicotinamide did lead to a significant improvement of 8% in regional control rate. This finding is consistent with the results of a recent systematic review and meta-analysis of hypoxia modification in head and neck cancer in which an absolute risk reduction of 8% for loco-regional control was observed.[33]

The high rate of local and regional control rates in both treatment arms of the current trial and the potential to perform successful salvage surgery in case of recurrence explains the lack of benefit in overall survival, similar to what is observed after conventional radiotherapy with concurrent cisplatinum in the RTOG 91-11 study.[2]

In the current study, long-term swallowing function was similar for AR and ARCON. One year after treatment, swallowing was limited to soft foods or liquid in 9% and 7% of patients respectively. This is lower than the 23% reported in RTOG 91-11.[2] However, direct comparison is difficult because of potential bias, e.g. RTOG 91-11 included more supraglottic tumors. Furthermore, unlike the current study, planned neck dissection was performed in case of cN2a or cN2b disease. Neck dissection after chemoradiotherapy has been demonstrated to increase the risk of long-term dysphagia and laryngeal dysfunction.[34]

Recently published data from this study demonstrate that ARCON results in a high level of compliance and a toxicity profile, comparable to what is encountered after accelerated radiotherapy alone.[16,35] The 8% improvement in regional control and excellent larynx preservation rate support the evidence that ARCON can achieve a therapeutic gain. Probably, head and neck tumors at other subsites, where the laryngeal cartilage is not the dose-limiting organ, e.g. oropharynx, will profit more from ARCON, especially those with advanced nodal stage. The recently published results from a phase III trial with carbogen and nicotinamide for bladder cancer confirm the value of this hypoxia-modifying approach.[36] Radiotherapy with carbogen and nicotinamide produced a significant improvement in local relapse rate, bladder conservation rate and overall survival. The additional costs (carbogen, nicotinamide) of ARCON are very low, making it a cost-effective regimen.

The present study demonstrates the importance of a proper patient selection that is based on the mode of action of the treatment under investigation, as only patients with hypoxic tumors did profit from ARCON therapy and no gain was seen in patients with well-oxygenated tumors. This finding strongly supports the notion that assessment of the tumor oxygenation status provides a powerful selection tool for hypoxia-modifying treatment on an individual patient basis. The inclusion of

unselected patient populations may be an important reason for the modest improvements generally reported by studies employing hypoxic modification.[33,37] Further translational research linked to this study is currently investigating the predictive value of a hypoxia-associated gene signature.

Conclusion

The use of ARCON in stage II-IV laryngeal cancer produced a significant gain in regional control rate compared to AR, with similar acute and late toxicity. There was no difference in local control between the treatment arms. Translational research employing a hypoxia marker assay demonstrates that proper patient selection based on tumor biology is the key to the success of this approach.

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3

Acute toxicity profile and compliance to accelerated radiotherapy plus carbogen and nicotinamide for clinical stage T2-4 laryngeal cancer: results of a phase III randomized trial

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Abstract

Purpose

We report the acute toxicity profile and compliance from a randomized phase III trial comparing accelerated radiotherapy (AR) with accelerated radiotherapy plus carbogen and nicotinamide (ARCON) in laryngeal cancer.

Methods and Materials

From April 2001 to February 2008, 345 patients with cT2-4 squamous cell laryngeal cancer were randomized to AR (N=174) and ARCON (N=171). Acute toxicity was scored weekly until week 8 and every 2-4 weeks thereafter. Compliance to carbogen and nicotinamide was reported.

Results

Between both treatment arms (AR vs. ARCON) no statistically significant difference was observed for incidence of acute skin reactions (moist desquamation: 56% vs. 58%, p=0.80), acute mucosal reactions (confluent mucositis: 79 vs. 85%, p=0.14) and symptoms related to acute mucositis (severe pain on swallowing: 53% vs. 58%, p=0.37; nasogastric tube feeding: 28 vs. 28%, p=0.98, narcotic medicines required: 58% vs. 58%, p=0.97). There was a statistically significant difference in median duration of confluent mucositis in favor of AR (2.0 vs 3.0 weeks, p=0.01). There was full compliance with carbogen breathing and nicotinamide in 86% and 80% of the patients with discontinuation in 6% and 12%, respectively. Adjustment of anti-emesis prophylaxis was needed in 42% of patients.

Conclusion

With exception of a slight increase in median duration of acute confluent mucositis, the present data reveal a similar acute toxicity profile between both regimens and a good compliance with ARCON for clinical stage T2-4 laryngeal cancers. Treatment outcome and late morbidity will determine the real therapeutic benefit.

Introduction

In the early 1990s, the landmark trial conducted by the Department of Veterans Affairs Laryngeal Cancer Study Group has shown that functional larynx preservation strategies based on induction chemotherapy followed by definitive radiotherapy do not compromise survival when compared with initial total laryngectomy (1). In the subsequent randomized RTOG 91-11 trial, the two years larynx preservation rate in patients treated with radiotherapy and concurrent cisplatin (88%) differed significantly from patients given induction chemotherapy followed by radiotherapy (75%, $p=0.005$) or conventional fractionated radiotherapy alone (70%, $p<0.001$) (2). In the same period, a single institution study with accelerated radiotherapy and carbogen breathing and nicotinamide (ARCON) for stage III-IV laryngeal cancer was conducted. This approach yielded a 2-year local control rate of 92% (3). Because this rate was higher than any previous report in literature, a phase III trial was initiated to assess the magnitude of the therapeutic gain.

In head and neck cancer, tumor cell repopulation and tumor hypoxia are known factors determining radiation response. To counteract the clonogenic repopulation during therapy, overall duration of radiotherapy is reduced, generally by delivering several fractions a day (accelerated radiotherapy; AR). A way of limiting hypoxia is the inhalation of carbogen (98% O_2 + 2% CO_2) to decrease *diffusion*-limited hypoxia combined with nicotinamide, a vasoactive agent to decrease *perfusion*-limited hypoxia (4,5,6,7,8). The combination of both strategies is applied in ARCON. In a murine mammary carcinoma an enhancement ratio of 1.9 was obtained with doses close to those used clinically (9). In previous trials with head and neck cancer patients, feasibility and tolerability were found acceptable despite elevated radiation reactions in rapidly repopulating normal tissues (3,10,11). Confluent mucositis was observed in 95% of patients, however eventually restoration of the mucosal lining was complete in all cases (10). Non-compliance of carbogen breathing in 12% of patients was mainly due to discomfort with the breathing procedure, usually related to the respiratory stimulation caused by the carbon dioxide component (11). Nausea and vomiting were the most frequent side effects of nicotinamide and responsible for discontinuation of drug intake in 26% of patients (10). However adjustment of the nicotinamide dose and anti-emesis prophylaxis reduced the discontinuation rate to 10% (11). More recently, the early results of the phase III trial with carbogen and nicotinamide in locally advanced bladder cancer (BCON) were published showing no increase in radiation-induced morbidity in the experimental arm (12).

The Dutch multicenter ARCON trial randomized patients with cT2-4 laryngeal cancer between accelerated radiotherapy (AR) and accelerated radiotherapy plus carbogen and nicotinamide (ARCON). This paper reports on the compliance and toxicity to carbogen and nicotinamide and the early normal tissue radiation side-effects.

Material and methods

In- and exclusion criteria

Approval for the study was obtained from the Radboud University Nijmegen Medical Centre research Ethics Committee with ratification from each centre before start.

Eligibility was assessed by a multidisciplinary head and neck oncology team. Patients underwent a laryngoscopy under general anesthesia with biopsy taking and histological assessment of the primary tumor. Further staging consisted of CT- or MR-imaging of the larynx and neck with ultrasound guided fine needle aspiration of suspected nodes and chest X-ray.

All patients over the age of 18, WHO performance status ≤1 and with pathological confirmed squamous cell carcinoma of the larynx and the following clinical stage (TNM-classification, UICC 1997), were considered: cT2 glottic carcinoma with impaired cord mobility or subglottic extension, cT2 supraglottic carcinoma with invasion of the mucosa of the base of tongue or vallecula or invasion of the medial wall of the piriform sinus, cT3-4 glottic or supraglottic carcinoma and any N-stage but M0.

Written informed consent and completed quality of life questionnaire was obtained before randomization.

Exclusion criteria included prior or concurrent treatment for this tumor, severe stridor with impossibility for adequate debulking of airway, impaired renal and/ or hepatic function (creatinine > upper normal limit, ASAT/ ALAT > 1.5 times upper limit), use of nefrotoxic or anti-convulsant medication that could not be discontinued for the duration of the radiation treatment and a history of malignancy during the previous 5 years (with exception of basal cell carcinoma of the skin, carcinoma in situ of the cervix or superficial bladder cancer).

Randomization

Patients fulfilling enrollment criteria were centrally randomized by phone at the IKO (Integraal Kankercentrum Oost) trials office. Treatment arm assignments (AR vs. ARCON) were stratified for tumor site (glottis vs. supraglottic) and institution.

Treatment

Radiotherapy

A CT-scan in treatment position and immobilization device was used in the majority of the patients. The initial planning target volume (PTV1) contained the primary tumor with margins and the lymph node levels II, III and IV in case of cN0. Level V and VI were added to the PTV1 in case of cN1-3 and >1.0 cm subglottic extension, respectively (13). The boost planning target volume (PTV2) encompassed the primary tumor volume and the macroscopic involved lymph nodes. A total dose of 44 Gy in 22 daily fractions of 2 Gy was prescribed on the PTV1 followed by a boost dose of 24 Gy in twice daily fractions of 2 Gy with a minimum interval of 6 hours between the fractions. Because a decrease in the radiation tolerance was observed for cartilage and spinal cord in earlier studies, the total dose to the arytenoid cartilage and the spinal cord in the ARCON arm was limited to 64 and 40 Gy, respectively (14,15).

Carbogen

A gas mixture of 98% O_2 + 2% CO_2 was delivered through a closed breathing system. Either an anesthetic face mask with airtight seal or a scuba diving mouthpiece with a nasal clip was used (16,17). Carbogen breathing was administered 4 minutes prior and during irradiation of the macroscopic tumor locations.

Nicotinamide

A daily oral dose of 60 mg/kg nicotinamide was given 1-1.5 h before the irradiations either as capsules or dissolved in water or lemonade. During the bi-fractionation part of treatment, nicotinamide was given only before the first fraction of the day. To prevent nausea, domperidone (10 mg, 3 dd) was given prophylactically, starting on the first day of treatment.

Follow-up and scoring of toxicity and acute adverse events

During the treatment and the two weeks thereafter, patients were evaluated weekly by the attending radiation oncologist. Depending on the severity of the acute radiation reactions, patients were seen every two to four weeks until healing of the reactions. Side-effects related to the skin (erythema, dry and moist desquamation) and the mucous membranes (mucositis and related symptoms like pain on swallowing, severity of dysphagia and required analgesics) were scored as shown in Table 1. Any other side-effects felt to be related to the carbogen/ nicotinamide administration and the reason for interruption or discontinuation were recorded as well. During the treatment haemoglobin, haematocrit, creatinine and urea level were checked weekly.

Table 1 Scores for skin and mucosal reactions.

Acute Reactions		Score
Skin		
erythema	none	0
	slight	1
	moderate	2
	severe	3
	unknown	9
dry desquamation	% of irradiated surface	
moist desquamation	% of irradiated surface	
Mucous membrane		
mucositis	none	0
	patchy	1
	confluent	2
	unknown	9
Symptoms related to acute mucositis		
pain on swallowing	none	0
	slight	1
	moderate	2
	severe	3
	not known	9
severity of dysphagia	none	0
	some discomfort on swallowing, no disturbance on diet	1
	difficulty with diet, soft diet required	2
	considerable difficulty on swallowing, fluids only	3
	severe difficulty on swallowing	4
	nasogastric tube or i.v. feeding	5
	not known	9
analgesics required	none	0
	topical medicine only	1
	non-narcotic medicines necessary	2
	narcotic medicines required	3
	not known	9

Statistics

Chi-square tests were carried out to evaluate differences in prevalence between the groups. Kaplan-Meier estimates were used to analyse the duration of the symptoms.

Results

Between April 2001 and February 2008, 345 patients with a histological diagnosis of squamous cell carcinoma of the larynx were randomized to either AR or ARCON in 7 university hospitals within the Netherlands and the U.K. One patient (ARCON) was excluded from analysis because of policy change after randomization. Demographic details are presented in table 2. There are no significant differences between the two study arms.

Acute skin and mucosal reactions and related symptoms

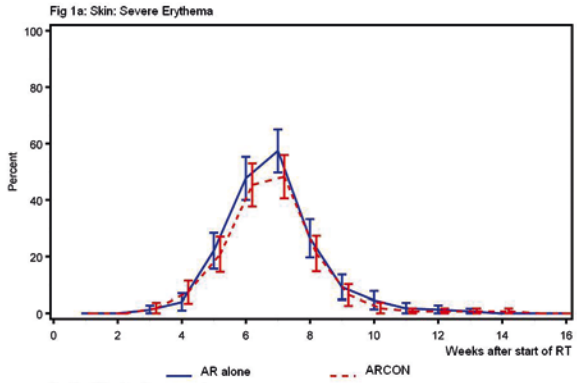
In figure 1 the evolution of severe erythema, dry and moist desquamation of the skin over the first 16 weeks of treatment is presented. No statistically significant difference was observed for occurence of severe erythema (65% vs. 63%, p=0.70), dry desquamation (78% vs. 73%, p=0.31) and moist desquamation (56% vs. 58%, p=0.80) with AR and ARCON, respectively. Between the groups, there was no difference in median duration of dry desquamation (AR vs. ARCON: 3.0 vs. 3.0 weeks; p=0.78) or moist desquamation (AR vs. ARCON: 1.5 vs. 2.0 weeks; p=0.59). Peak incidence of skin reactions in both study arms was seen in the first week after the end of radiotherapy (week 7).

Figure 2 shows the incidence over time of confluent mucositis and related symptoms like severe pain on swallowing, nasogastric tube feeding and narcotic medicines required. With a peak incidence in the first week after the end of radiotherapy, no statistically significant difference was observed between AR and ARCON in occurrence of confluent mucositis (79 vs. 85%, p=0.14), severe pain on swallowing (53% vs. 58%, p=0.37), nasogastric tube feeding (28 vs. 28%, p=0.98) or narcotic medicines required (58% vs. 58%, p=0.97). There was a statistically significant difference in median duration of confluent mucositis in favor of AR (2.0 vs. 3.0 weeks, p=0.03). At 8 weeks after treatment, healing of patchy and confluent mucositis was complete in 87% and 84% (p=0.39) of the patients in the AR group and the ARCON group. At this time point tube feeding and narcotics were still required in 6 vs. 5% (p=0.85) and 9 vs. 8% (p=0.90) of the AR and ARCON patients, respectively.

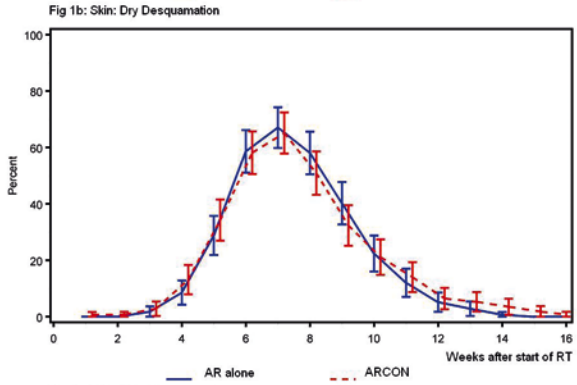
Table 2 Characteristics of patients, grouped by treatment regimen received.

Characteristics	Patients (N)	AR (N= 174)	ARCON (N= 170)
Age (years)			
Median		60	61
Range		38-88	41-84
Sex no (%)			
male		136 (78%)	141 (83%)
female		38 (22%)	29 (17%)
Site of the primary tumor (%)			
Supraglottic		100 (57%)	96 (56%)
Glottic		74 (43%)	74 (44%)
T-stage (%)			
T2		67 (38%)	55 (32%)
T3		80 (46%)	95 (56%)
T4		27 (16%)	20 (12%)
N-stage (%)			
N0		117 (67%)	116 (68%)
N1		20 (12%)	22 (13%)
N2a		4 (2%)	7 (4%)
N2b		10 (6%)	5 (3%)
N2c		23 (13%)	20 (12%)
N3		0 (0%)	0 (0%)
Participating Institutions			
Radboud University Nijmegen Medical Centre, Nijmegen	151	75	76
University Medical Center Utrecht, Utrecht	77	38	39
VU University Medical Center, Amsterdam	40	21	19
University Medical Center Groningen, Groningen	28	15	13
Maastricht University Medical Centre, Maastricht	28	15	13
Leiden University Medical Center, Leiden	17	8	9
Mount Vernon Hospital, Northwood, UK	3	2	1

a



b



c

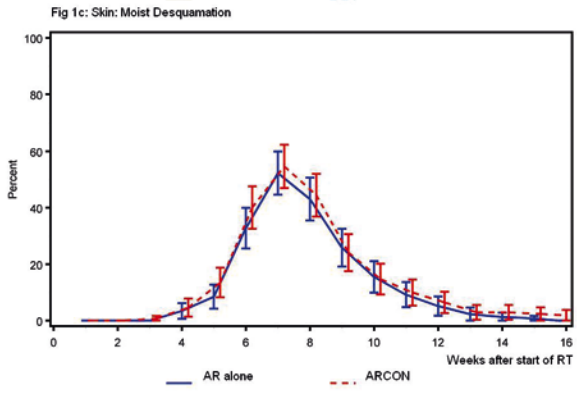
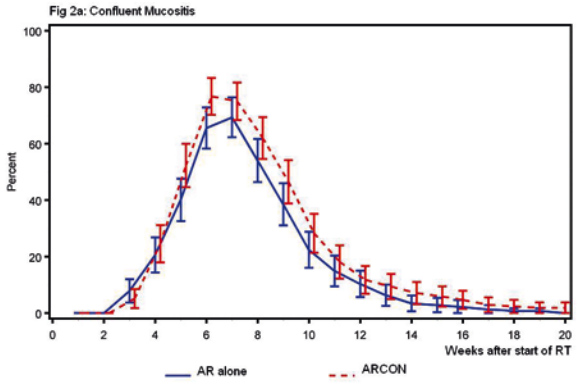


Figure 1 Evolution of occurrence of severe erythema (**a**), dry (**b**) and moist (**c**) desquamation with accelerated radiotherapy alone (solid line) or with ARCON (dotted line).

a



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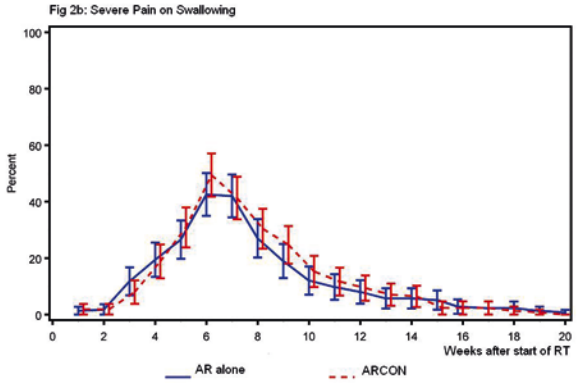

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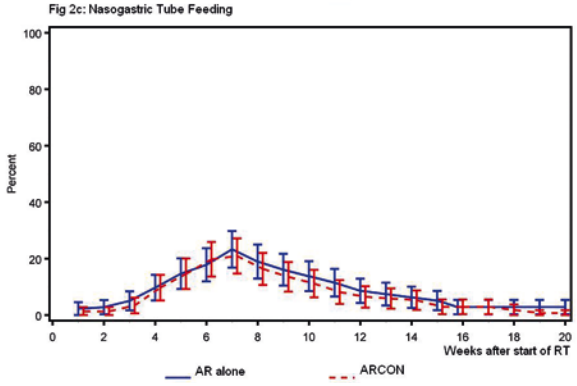

Figure 2 Evolution of confluent mucositis (**a**) and related symptoms like severe pain on swallowing (**b**), nasogastric tube/intravenous feeding (**c**) and need of narcotic medicines (**d**) with accelerated radiotherapy alone (solid line) or ARCON (dotted line).

d

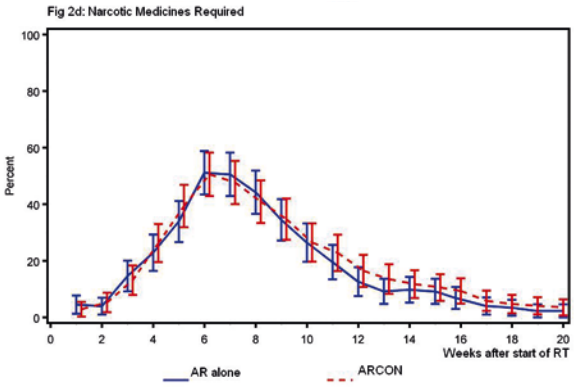

Figure 2 Continued.

Compliance to radiotherapy

Radiotherapy was delivered as planned to 173 (99%) AR and 169 (99%) ARCON patients and was completed within the specified time of 38 days for 168 (97%) AR and 163 (96%) ARCON patients. Patients with a longer overall treatment time had a mean delay of 3 days (range 1-7) and 2 days (range 1-5) for AR and ARCON, respectively.

One patient (AR) died from a cardiac arrest during treatment. The cause of death was unrelated to treatment. Another patient (ARCON), with full compliance to carbogen breathing and nicotinamide intake, died one day after the end of treatment due to a gastric bleeding. Shortly before he started diclofenac, known to cause gastric irritation, for abdominal pain. Causality with nicotinamide was considered uncertain for this patient. Upper airway obstruction during radiotherapy, requiring tracheostomy, occurred in 4 patients treated by AR and in 1 patient with ARCON. In the experimental arm one patient developed a renal insufficiency and sepsis and one patient was admitted to the hospital for severe blood spitting based on a tumoral bleeding.

Compliance and acute toxicity to nicotinamide and carbogen

The compliance to carbogen breathing was high (N=147/170; 86%) with a temporary interruption for a mean period of 2 days (range 1-4) in 12 patients (7%) and discontinuation in 11 patients (6%). The main reasons for discontinuation were discomfort with the breathing procedure (N=8), cardio-pulmonary co-morbidity (N=2) and stridor (N=1).

The majority of the patients fully complied with nicotinamide intake on all days radiotherapy was given (N=136/170; 80.0%). There was a temporary interruption in 14 patients (8%) for a mean period of 3 days (range 1-20) and discontinuation in 20 patients (12%). The main reasons for nicotinamide discontinuation were nausea and vomiting despite domperidon use (N=7), combined discontinuation with carbogen breathing (N=7), renal function disturbances (N=2), allergic reaction (N=1) and other reasons (N=3). Domperidon dose adjustment and/ or supplementary anti-emetics were needed in 72 patients (42%).

Discussion

The results of this randomized trial, comparing accelerated radiotherapy alone with accelerated radiotherapy in combination with carbogen and nicotinamide, reveal a good compliance with ARCON and an almost identical acute toxicity profile compared to AR for clinical stage T2-4 laryngeal cancers.

Although previous phase I en II head and neck clinical trials do suggest an enhancement of acute reactions on normal tissues by a hypoxia reducing regimen, early skin and mucosal reactions seem to be equally toxic. A likely explanation for this observation is the lower boost dose (64 vs. 68 Gy) given to patients treated with ARCON in the current study. This dose reduction was based on a decrease in radiation tolerance in the order of 10% observed for cartilage when radiotherapy was given in hyperbaric oxygen (14). The purpose was to arrive at equal late laryngeal toxicity in both study arms. The equal acute mucosal toxicity can be the result of this dose difference as well.

In contrast to observations in mouse skin in which enhancement ratios of 1.2 to 1.36 were estimated for early skin reactions, no difference in occurrence and median duration of severe erythema, dry or moist desquamation was observed between both arms (18,19). In a previous study with accelerated radiotherapy alone, 50% of patients develop a moist desquamation (20). When carbogen and nicotinamide are added, there is a further increase in the range of 57-65% for a median duration of 2 weeks (3,10,11). In the current study, no differences between AR and ARCON are observed, however results (56% and 58% for AR and ARCON) still fit within the range of previous data.

The early reacting tissue of greater clinical relevance remains the mucosal membrane. Acceleration alone up to the same dose causes confluent mucositis in up to 90% of laryngeal cancer patients with complete healing within 6 weeks after completion in all

18 cases (20). Combined with ARCON, confluent mucositis occurred in 91-97% and lasted for a median duration of 6 weeks with a of range 1-16 weeks (3,10,11). The further enhancement of the mucosal reactions by ARCON was especially reflected by a delayed recovery with prolonged healing. Strikingly, this confluent mucositis rate was less pronounced in the current trial and although a statistical significant increase in median duration is observed, a confluent mucositis period of only 3 weeks (range 0-20 weeks) is still half of that observed in previous trials.

Given the mild toxicity profile of the current study compared with our previous studies and the large number of patients enrolled from Nijmegen (N=144), a subgroup analysis was performed (3,10,11). A significantly higher occurrence of confluent mucositis (AR & ARCON: 92% & 93% vs. 71% & 79%, $p<0.01$) and median duration of confluent mucositis (AR & ARCON: 3.0 & 5.0 weeks vs. 2.0 & 2.0 weeks, $p<0.01$) was observed for patients treated in Nijmegen compared to the other centers. These interesting findings suggest that, despite the fact that the same toxicity scale with objective criteria is used, there can be significant inter-observer and inter-institutional variations in toxicity scoring. It is of importance to be aware of this phenomenon when interpreting data from multicenter studies. This is probably of even greater relevance for late toxicity as there is general suspicion that there is underestimation of the incidence and severity of late sequelae, especially in combined modality (chemoradiation) trials (21).

Severe pain due to mucositis and associated supportive care like (naso)gastric tube feeding did not differ significantly between the two treatment arms. Although the use of opioids and tube feeding are subjective parameters and potentially dependent on institution protocols, the 28% rate of tube feeding for the whole group is in line with the 25-34% observed in the earlier studies (3,10,11).

The compliance rate for carbogen breathing was 86% and is in the range of the Bladder ARCON trial (85%) recently published by Hoskin et al. (12). The rationale for use of gases that include a small fraction of carbon dioxide added to oxygen is an increase in respiratory drive and better blood perfusion due to the carbon-dioxide induced vasodilatation (22,23,24). The net result of carbogen breathing is a significant increase in tumor oxygenation as was demonstrated earlier by measurements in tumor bearing mice and in head-and neck cancer patients (6,7,8,22). Micro-electrode measurements of tissue pO_2 consistently indicated improved tumor oxygenation with carbon dioxide fractions between 1% and 5% with no significant differences between the fractions (22). Subsequently, a gas mixture of 98% O_2 and 2% CO_2 was chosen for the current trial. This resulted in a discontinuation rate of only 6% of patients and is better than the previous reported data of 12% and 16% with carbon dioxide

concentrations of 5% (3,11). The main reason for discontinuation was discomfort with the breathing procedure.

With 80% of patients receiving 60 mg/kg nicotinamide intake on all days radiotherapy was given and discontinuation in only 12% of patients, compliance can be regarded as good. Dose adjustments of domperidon and/ or supplementary use of anti-emetics were needed in 42% of patients. The potential digestive side-effects may be caused by a reduction in peristalsis due to the interaction of nicotinamide with the gastrointestinal smooth muscle in a dose-dependent matter (25). Comparative-dose studies of mice and humans led to the calculation that, for the radiosensitising effect in humans, a nicotinamide plasma concentration of 700 μmol/L was required, which is obtained with an oral dose of 80 mg/kg (26,27). However, daily administration of this dose during 5 to 7 weeks resulted in discontinuation of nicotinamide intake in over 25% of patients (10,11,28). Subsequent laser Doppler-flow measurements in patients still demonstrated a significant increase in blood flow during inhalation of carbogen with nicotinamide doses of 80 mg/kg and 40 mg/kg compared to carbogen alone (29). With a reduction in dose to 60 mg/kg and domperidone as antiemetic prophylaxis, the rate of discontinuation was significantly improved and in the range of the current results (3,11,30). In the bladder BCON trial, only 59% of patients fully complied despite anti-emetic prophylaxis and a further 9% completed the treatment when the dose was reduced to 40 mg/kg (12). This inferior compliance compared to the current trial can probably be explained by the concomitant partial abdomen irradiation.

Conclusion

The result of this phase III randomized trial reveals a similar acute toxicity profile and a good compliance with ARCON compared to AR for clinical stage T2-4 laryngeal cancers. Although analysis of late toxicity is still ongoing, the current observations provide a convenient base for determining the real therapeutic benefit of ARCON.

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4

Improved recurrence-free survival with ARCON for anemic patients with laryngeal cancer

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Abstract

Purpose

Anemia is associated with poor tumor control. It was previously observed that accelerated radiotherapy combined with carbogen breathing and nicotinamide (ARCON) can correct this adverse outcome in patients with head and neck cancer. The purpose of this study was to validate this observation based on data from a randomized trial.

Experimental design

Of 345 patients with cT2-4 laryngeal cancer, 174 were randomly assigned to accelerated radiotherapy (AR) and 171 to ARCON. Hemoglobin (Hb)-levels, measured before treatment, were defined as low when $<$7.5 mmol/L for women and $<$8.5 mmol/L for men. The hypoxia marker pimonidazole was used to assess the oxygenation status in tumor biopsies. Data were analyzed two years after inclusion of the last patient.

Results

Pre-treatment Hb-levels were available and below normal in 27/173 (16%) AR and 27/167 (16%) ARCON patients. In patients with normal pre-treatment Hb-levels treatment with ARCON had no significant effect on 5-year loco-regional control (LRC, 79% vs 75%, $P=0.44$) and disease-free survival (DFS, 75% vs 70%, $P=0.46$) compared to AR. However, in patients with low pre-treatment Hb-levels ARCON significantly improved 5-year LRC (79% vs 53%, $P=0.03$) and DFS (68% vs 45%, $P=0.04$). In multivariate analysis including other prognostic factors, pre-treatment Hb remained prognostic for LRC and DFS in the AR treatment arm. No correlation between pre-treatment Hb-levels and pimonidazole uptake was observed.

Conclusion

Results from the randomized phase 3 trial support previous observations that ARCON has the potential to correct the poor outcome of anemic cancer patients.

Introduction

Up to 40% of patients with solid tumors undergoing radiotherapy are anemic at presentation (1). A wealth of data indicate that low pre-treatment hemoglobin (Hb) levels are a strong prognostic indicator of poor disease control and survival (2,3,4). To overcome the adverse impact of low pre-treatment Hb-levels, correction strategies have been applied such as administering erythropoietin and packed cell transfusions. However, randomized trials failed to demonstrate the effectiveness of these approaches in patients with head and neck or breast cancer, and erythropoietin was even counterproductive in some studies (3,5,6,7).

Disappearance of the adverse impact of anemia was observed in a non-randomized phase II trial when accelerated radiotherapy was combined with carbogen breathing and nicotinamide (ARCON) (8). This strategy counteracts tumor cell repopulation and hypoxic radioresistance (9). Carbogen breathing (98% O_2 + 2% CO_2) is used to decrease diffusion-limited hypoxia while nicotinamide, a vasoactive agent, is added to limit hypoxia caused by intermittent reduction of blood flow (9). The same regimen has subsequently been studied in a randomized phase III trial, comparing accelerated radiotherapy alone (AR) or combined with carbogen and nicotinamide (ARCON) for locally advanced laryngeal cancers. Although local control is not improved, it was demonstrated that ARCON results in a benefit of 8% in regional control and excellent larynx preservation rates without increase of toxicity (10,11). Translational research employing the hypoxia marker pimonidazole, demonstrated that proper patient selection based on tumor biology is the key to the success of this approach.

Based on the results of the preceding phase II study the hypothesis was generated that ARCON can abolish the adverse impact of anemia (8). The purpose of the current study is to find support for this observation in the data from the randomized phase III study. This paper reports on the impact of ARCON on the prognosis of a cohort of larynx cancer patients presenting with pre-treatment anemia.

Material and methods

Study Design and Eligibility

This was an open-label, randomized phase III trial comparing AR with ARCON in patients with cT2-4 laryngeal cancer. The trial (ClinicalTrials.gov NCT00147732) was conducted under the auspices of the Dutch Head and Neck Cancer Group in 7 centers in the Netherlands and the UK (Table S1). Eligibility criteria are provided in the supplementary Table S2.

Approval for the study was obtained from the Radboud University Nijmegen Medical Centre Research Ethics Committee with ratification from each center. Written informed consent was obtained before randomization.

Randomization

Patients were centrally randomized by phone at the IKO (Integraal Kankercentrum Oost) trials office. Treatment arm assignments (AR vs ARCON) were stratified for tumor site (glottic vs supraglottic) and institution. A dynamic allocation method was used to avoid imbalance of treatment assignment within an institution. Randomization took place after all study investigations and no longer than 4 weeks prior to the anticipated start of treatment.

Procedure

A radiation dose of 44 Gy in 22 daily fractions of 2 Gy was prescribed to the primary tumor and neck nodes followed by a boost dose of 24 Gy in twice daily fractions of 2 Gy to the primary tumor and involved lymph nodes. Because a decrease in the radiation tolerance was observed for cartilage and spinal cord in earlier studies with hypoxic sensitization, the total dose to the arytenoid cartilage and the spinal cord in the ARCON arm was limited to 64 and 40 Gy, respectively (12,13).

Patients allocated to the ARCON arm received carbogen (98% O_2 + 2% CO_2, 4 minutes before and during radiotherapy) and nicotinamide (60 mg/kg, 1-1.5 hours before fractions) concurrently with radiotherapy. Details of the procedure are described previously (10,11).

All patients participating in this study were treated in academic hospitals with accreditation in head and neck oncology by the Dutch Cooperative Head and Neck Oncology Group and institution-wide quality assurance programs.

Hypoxia marker analysis

After additional informed consent, patients received pimonidazole (Hypoxyprobe-1; Natural Pharmacia International, Belmont, MA) intravenously (500 mg/m^2) two hours before biopsy taking. Biopsies were snap frozen in liquid nitrogen, immunohistochemically stained and semi-automatically analyzed (14). Of each biopsy, one complete section was analyzed for the hypoxic fraction, i.e. the tumor area positive for pimonidazole relative to the total tumor area.

Monitoring During Treatment and Follow-up Evaluations

Before and weekly during treatment hemoglobin, hematocrit, creatinine and urea levels were obtained. Normal Hb-levels were defined as: 7.5-10 mmol/L for women

and 8.5-11 mmol/L for men (women: 12-16 g/dl; men: 13.6-17.7 g/dl). Follow-up visits took place every 2, 3, 4 months during the first, second and third year respectively, then every 6 months for another 2 years. The larynx was assessed by fiberoptic or indirect laryngoscopy. Regional control was assessed by palpation of the neck. When tumor recurrence was suspected, imaging (CT-scan or MRI) was performed to document the extent of the disease and biopsies were taken for pathological confirmation.

Endpoints and Statistics

Survival endpoints used were loco-regional control (LRC), metastasis-free survival (MFS), disease-free survival (DFS) and overall survival (OS) at 5 years from randomization. All intervals were calculated from the date of randomization and censored after 60 months or at last follow-up. LRC was defined as freedom of first recurrence at the primary tumor site and complete and persistent disappearance of the pathological lymph nodes after radiotherapy, not including salvage procedures. MFS was defined as the time from randomization to distant metastasis. DFS was defined as the time to local or regional recurrence, or distant metastasis. OS was defined as time to death.

The primary endpoint of the randomized trial was local control. In the AR arm a local control rate of 60% at 2 years after completion of radiotherapy was expected. An improvement by 15%, resulting in a local control rate of 75% at 2 years, was assumed for the ARCON arm. In order to detect this difference of 15% with a significance level of 0.05 and a power of 0.80 (two-sided log-rank test), 156 patients were required in each treatment group. To account for a drop out percentage of 10%, an extra 16 patients were added for each group.

Statistical analyses were performed using SPSS 19.0.0. Mann-Whitney U and Chi-square tests were used at a two-sided significance level of 0.05. Endpoints were evaluated by the Kaplan-Meier method based on intent-to-treat policy and compared with log-rank testing. A multivariate Cox proportional hazards analysis, with stepwise backward elimination of variables at $P>0.1$, was used for both patient groups and included N-classification (N+ vs N0), performance status (0 vs 1) and treatment arm (ARCON vs AR). An interaction between treatment arm and pre-treatment Hb-level was assessed using Cox regression.

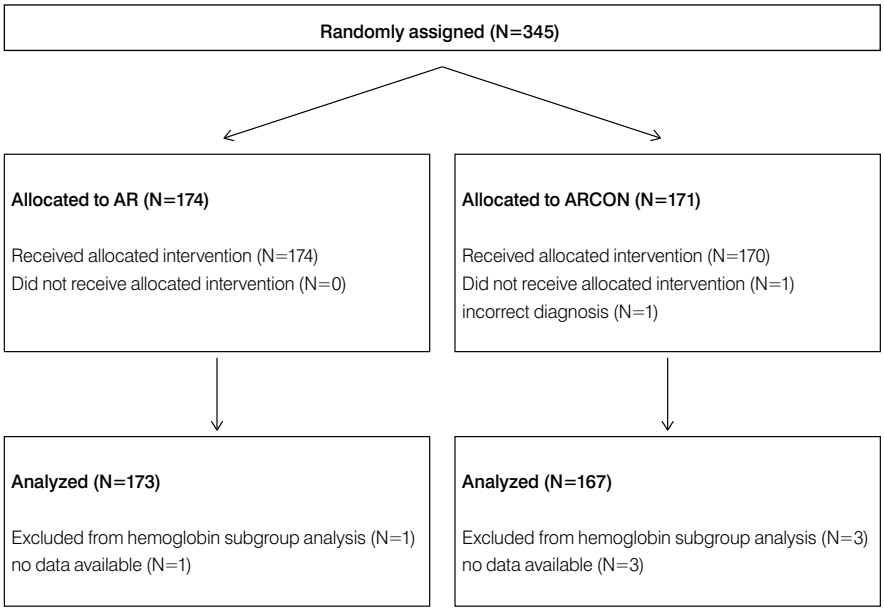
Role of the funding source

The study sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Patient Characteristics and Protocol Compliance

Between April 2001 and February 2008, 345 patients were randomized to either AR (n=174) or ARCON (n=171) (Figure 1). The median follow-up time was 44 (range 18-103), 55 and 60 months for the whole group and for patients still alive receiving AR and ARCON, respectively. Patient demographics and clinical tumor characteristics were well balanced without significant differences within the whole study population and the group of patients presenting with anemia (Table 1). Patients presenting with anemia were older (P=0.01), had a poorer performance status (P<0.01), more N2 stages (P<0.01) and higher stage grouping (P=0.02) compared to the whole group of patients. Compliance to radiotherapy, carbogen breathing and nicotinamide intake was high and comparable between both groups. Detailed information is listed in the supplementary Table S3.



AR: Accelerated Radiotherapy
ARCON: Accelerated Radiotherapy with Carbogen and Nicotinamide

Figure 1 CONSORT diagram.

Table 1 Demographics and clinical characteristics for all patients (N=345) and patients presenting with anemia (N=54).

Characteristics	All patients			Anemic patients		
	AR (N=174)	ARCON (N=171)	*P*	AR (N=27)	ARCON (N=27)	*P*
Age - yr			0.56[a]			0.48[a]
Median	60	61		63	64	
Range	38-88	41-84		48-76	43-80	
Sex - no. (%)			0.16[b]			0.10[b]
Male	136 (78)	142 (83)		22 (81)	26 (96)	
Female	38 (22)	29 (17)		5 (19)	1 (4)	
Performance status - no. (%)			0.54[b]			0.43[b]
0	140 (80)	137 (81)		16 (59)	17 (63)	
1	34 (20)	34 (19)		11 (41)	10 (37)	
Site of the primary tumor - no. (%)			0.49[b]			0.50[b]
Supraglottic	100 (57)	97 (56)		17 (63)	17 (63)	
Glottic	74 (43)	74 (44)		10 (37)	10 (37)	
AJCC stage groupings - no. (%)			0.35[b]			0.07[b]
Stage II	53 (31)	46 (27)		9 (33)	4 (15)	
Stage III	65 (37)	77 (45)		4 (15)	11 (41)	
Stage IV	56 (32)	48 (28)		14 (52)	12 (44)	
T-stage - no. (%)			0.20[b]			0.12[b]
T2	67 (38)	55 (32)		13 (48)	6 (22)	
T3	80 (46)	95 (56)		11 (41)	15 (56)	
T4	27 (16)	21 (12)		3 (11)	6 (22)	
N-stage - no. (%)			0.58[b]			0.29[b]
N0	117 (67)	116 (68)		15 (56)	17 (63)	
N1	20 (12)	23 (13)		0 (0)	2 (7)	
N2a	4 (2)	7 (4)		2 (7)	3 (11)	
N2b	10 (6)	5 (3)		2 (7)	0 (0)	
N2c	23 (13)	20 (12)		8 (30)	5 (19)	
N3	0 (0)	0 (0)		0 (0)	0 (0)	

Table 1 Continued.

Characteristics	All patients			Anemic patients		
	AR (N=174)	ARCON (N=171)	*P*	AR (N=27)	ARCON (N=27)	*P*
Pre-treatment Hemoglobin - no. (%)			0.49[b]			
Normal	146/173 (84)	140/167 (84)		0/27 (0)	0/27 (0)	
Low	27/173 (16)	27/167 (16)		27/27 (100)	27/27 (100)	
Male mmol/L						
Median	9.2	9.1		8.0	8.1	
Range	6.1-14.5	6.1-13.4		6.1-8.4	6.1-8.4	
Female mmol/L						
Median	8.4	8.3		7.3	7.2	
Range	5.8-11.0	7.2-9.9		5.8-7.4	7.2-7.2	

[a] Mann-Withney U test
[b] Chi-Square test

Hemoglobin levels

Pre-treatment Hb-levels were available and below normal in 27/173 (16%) AR and 27/167 (16%) ARCON patients (Table 1). Four of them (AR n=3; ARCON n=1) received a transfusion, given as two units of packed red blood cells. A correlation was observed between low Hb at presentation and lower performance status ($P<0.01$), and a trend was observed between low Hb and higher N-status ($P=0.06$).

Loco-regional control and survival

In patients with normal pre-treatment Hb-levels treatment with ARCON had no significant effect on 5-year LRC (79% vs 75%, $P=0.44$) and DFS (75% vs 70%, $P=0.46$) compared to AR. However, in patients with low pre-treatment Hb-levels ARCON treatment significantly improved 5-year LRC (79% vs 53%, $P=0.03$) and DFS (68% vs 45%, $P=0.04$) (Figure 2A, 2B). No significant benefit of ARCON was observed for MFS (normal Hb: ARCON vs AR: 91% vs 89%, $P=0.56$; low Hb: ARCON vs AR 87% vs 69%, $P=0.10$; Figure 3A). Patients presenting with low Hb-levels had a worse 5-year OS regardless of the treatment regimen (normal Hb: ARCON vs AR: 65% vs 65%, $P=0.93$; low Hb: ARCON vs AR 46% vs 28%, $P=0.97$; Figure 3B).

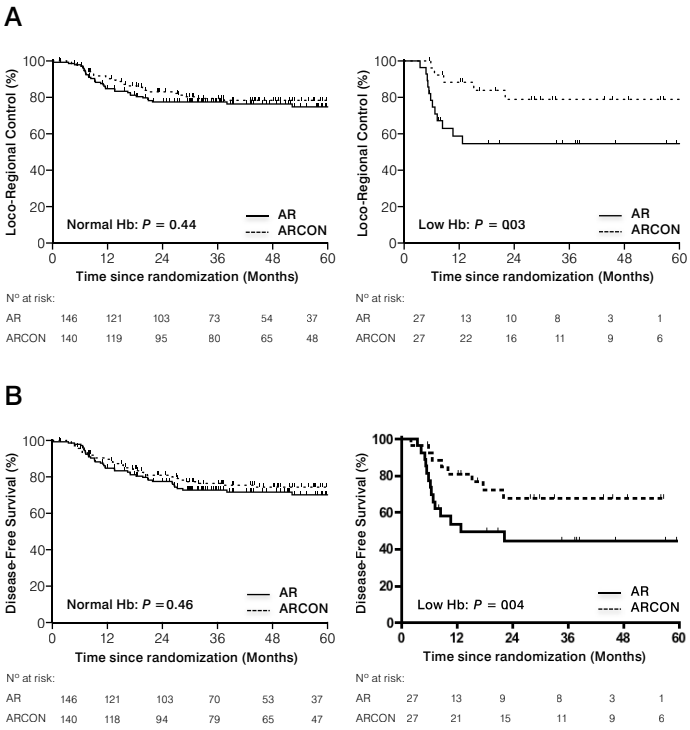


Figure 2 A-B. Loco-regional control (2A) and disease-free survival (2B) for patients with low and normal pre-treatment hemoglobin levels, treated by AR or ARCON.

Prognostic factors for tumor control

The impact of various common prognostic factors and Hb-levels on LRC, MFS, DFS and OS is summarized in Table 2**.** On multivariate analysis after correction for N-stage and Hb-level, ARCON treatment remained an independent prognostic factor for LRC ($P=0.04$) and DFS ($P=0.09$) in anemic patients only. A significant interaction between treatment effect and Hb-level was found for LRC ($P=0.02$) and DFS ($P=0.05$).

Hypoxia marker study

Tumor biopsies of 79 patients were available for pimonidazole staining. Characteristics were well balanced between patients receiving AR and ARCON (Table 3). However, the group of patients participating in the hypoxia marker study differed in some aspects from the entire study population: patients involved were more frequently female ($P<0.01$) and presented with higher tumor stage ($P<0.01$).

Table 2 Univariate and multivariate analysis per Hb levels.

		Normal Hb (N=286)		Low Hb (N=54)	
		HR (95% CI)	*P*	HR (95% CI)	*P*
		Loco-regional Control			
Univariate parameter					
Age	>60 vs ≤60	0.94 (0.57-1.55)	0.81	0.47 (0.18-1.22)	0.12
Sex	Female vs Male	0.30 (0.12-0.76)	0.01	1.31 (0.30-5.77)	0.72
Site	Supraglottic vs Glottic	0.81 (0.49-1.33)	0.40	1.45 (0.51-4.13)	0.48
T-classification	T2 vs T3-4	0.85 (0.52-1.42)	0.54	0.99 (0.37-2.67)	0.98
N-classification	N+ vs N0	1.85 (1.12-3.06)	0.02	2.91 (1.10-7.68)	0.03
Performance Status	0 vs 1	0.58 (0.25-1.34)	0.20	1.68 (0.65-4.35)	0.29
Treatment	ARCON vs AR	0.82 (0.49-1.35)	0.43	0.31 (0.11-0.89)	0.03
Multivariate analysis					
N-classification	N+ vs N0	1.86 (1.12-3.06)	0.02	3.17 (1.19-8.42)	0.02
Performance Status	0 vs 1	0.60 (0.26-1.39)	0.23	1.91 (0.72-5.06)	0.19
Treatment	ARCON vs AR	0.80 (0.48-1.33)	0.39	0.33 (0.11-0.93)	0.04
		Metastasis-Free Survival			
Univariate parameter					
Age	>60 vs ≤60	0.63 (0.28-1.44)	0.28	0.61 (0.17-2.16)	0.44
Sex	Female vs Male	0.70 (0.24-2.04)	0.51	1.07 (0.14-8.50)	0.95
Site	Supraglottic vs Glottic	1.95 (0.81-4.71)	0.14	1.26 (0.32-4.85)	0.74
T-classification	T2 vs T3-4	1.47 (0.61-3.54)	0.40	0.86 (0.24-3.07)	0.82
N-classification	N+ vs N0	4.80 (2.06-11.22)	<0.01	2.82 (0.79-10.06)	0.11
Performance Status	0 vs 1	1.51 (0.56-4.05)	0.41	1.78 (0.51-6.14)	0.37
Treatment	ARCON vs AR	0.78 (0.34-1.76)	0.55	0.35 (0.09-1.33)	0.12
Multivariate analysis					
N-classification	N+ vs N0	4.80 (2.06-11.22)	<0.01	3.31 (0.95-11.5)	0.06
Performance Status	0 vs 1	1.78 (0.66-4.78)	0.26	1.63 (0.49-5.39)	0.43
Treatment	ARCON vs AR	0.74 (0.33-1.66)	0.46	0.41 (0.11-1.55)	0.19

		Normal Hb (N=286)		Low Hb (N=54)	
		HR (95% CI)	*P*	HR (95% CI)	*P*
			Disease-free Survival		
Univariate parameter					
Age	>60 vs ≤60	0.90 (0.57-1.43)	0.65	0.55 (0.23-1.30)	0.17
Sex	Female vs Male	0.42 (0.20-0.87)	0.02	1.06 (0.25-4.59)	0.94
Site	Supraglottic vs Glottic	1.02 (0.64-1.62)	0.93	1.59 (0.61-4.09)	0.34
T-classification	T2 vs T3-4	0.93 (0.58-1.50)	0.77	0.92 (0.38-2.22)	0.85
N-classification	N+ vs N0	2.20 (1.39-3.48)	<0.01	2.98 (1.25-7.12)	0.01
Performance Status	0 vs 1	0.86 (0.44-1.69)	0.67	1.39 (0.58-3.30)	0.46
Treatment	ARCON vs AR	0.84 (0.53-1.33)	0.45	0.44 (0.18-1.05)	0.06
Multivariate analysis					
N-classification	N+ vs N0	2.20 (1.39-3.48)	<0.01	3.56 (1.49-8.50)	<0.01
Performance Status	0 vs 1	0.91 (0.46-1.77)	0.77	1.44 (0.61-3.42)	0.41
Treatment	ARCON vs AR	0.82 (0.51-1.30)	0.40	0.47 (0.20-1.13)	0.09
			Overall Survival		
Univariate parameter					
Age	>60 vs ≤60	1.54 (1.03-2.30)	0.04	1.08 (0.48-2.43)	0.86
Sex	Female vs Male	0.74 (0.44-1.24)	0.25	1.84 (0.54-6.20)	0.33
Site	Supraglottic vs Glottic	1.18 (0.79-1.76)	0.41	0.82 (0.38-1.76)	0.61
T-classification	T2 vs T3-4	1.29 (0.85-1.97)	0.23	1.24 (0.57-2.68)	0.59
N-classification	N+ vs N0	1.66 (1.12-2.48)	0.01	1.81 (0.87-3.77)	0.11
Performance Status	0 vs 1	1.99 (1.25-3.16)	<0.01	1.44 (0.67-3.09)	0.35
Treatment	ARCON vs AR	1.02 (.69-1.51)	0.93	0.99 (0.47-2.05)	0.97
Multivariate analysis					
N-classification	N+ vs N0	1.17 (1.03-1.33)	0.02	1.22 (0.99-1.50)	0.06
Performance Status	0 vs 1	2.04 (1.29-3.25)	<0.01	1.56 (0.72-3.37)	0.26
Treatment	ARCON vs AR	1.05 (0.70-1.55)	0.83	1.18 (0.56-2.47)	0.67

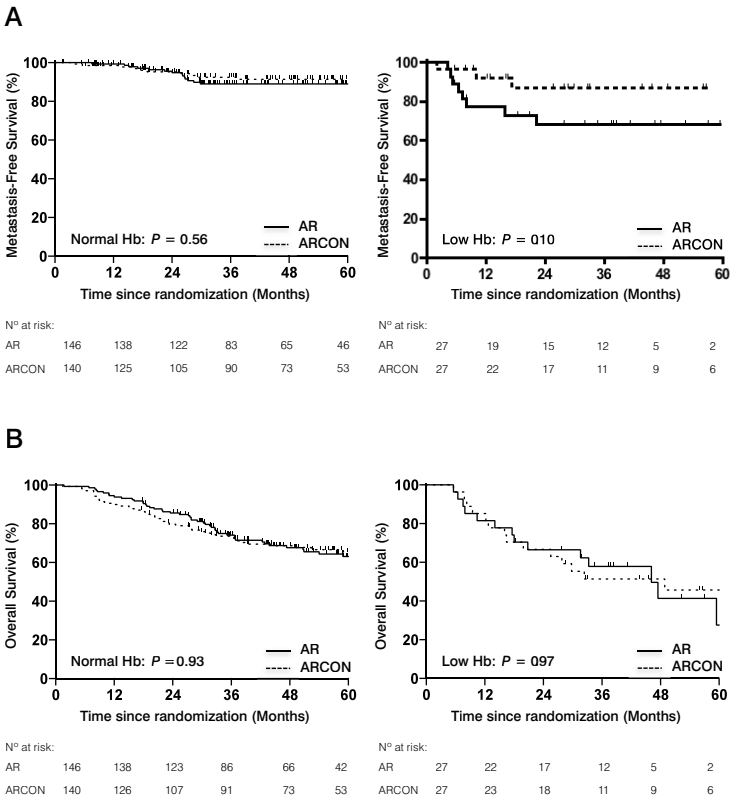


Figure 3 A-B. Metastasis-free survival (3A) and overall survival (3B) for patients with low and normal pre-treatment hemoglobin levels, treated by AR or ARCON.

The hypoxic fraction, as defined by pimonidazole staining, varied from 0-19.4% with a median value of 1.6%. Eleven of the 79 patients had Hb-levels below normal at diagnosis. No correlation was observed between pre-treatment Hb-levels and hypoxic fraction (P=0.11).

Table 3 Demographics and clinical characteristics of patients (N=79) participating in the hypoxia marker study.

Characteristics	AR (N=38)	ARCON (N=41)	*P*
Age - yr			0.41[a]
Median	61	61	
Range	38-81	46-83	
Sex - no. (%)			0.99[b]
Male	26 (68)	28 (68)	
Female	12 (32)	13 (32)	
Performance status - no. (%)			0.71[b]
0	29 (76)	34 (83)	
1	9 (24)	7 (17)	
Site of the primary tumor - no. (%)			0.99[b]
Supraglottic	25 (66)	27 (66)	
Glottic	13 (34)	14 (34)	
T-stage - no. (%)			0.16[b]
T2	11 (29)	6 (15)	
T3	20 (53)	30 (73)	
T4	7 (18)	5 (12)	
N-stage - no. (%)			0.24[b]
N0	17 (45)	20 (49)	
N1	10 (26)	5 (12)	
N2a	3 (8)	1 (2)	
N2b	1 (3)	4 (10)	
N2c	7 (18)	11 (27)	
N3	0 (0)	0 (0)	
Pre-treatment Hemoglobin - no. (%)			0.27[b]
Normal	31 (82)	37 (90)	
Low	7 (18)	4 (10)	
Male mmol/L			
Median	9.0	9.1	
Range	7.7-10.3	6.8-10.4	
Female mmol/L			
Median	7.9	8.2	
Range	7.4-9.3	7.5-9.9	

[a] Mann-Withney U test
[b] Chi-Square test

Discussion

It has been demonstrated by numerous reports that anemia in patients with cancer of the cervix, head and neck, bladder, breast and lung is associated with poor outcome (2,3,4). This is also found in the current study for patients with T2-4 laryngeal cancer treated with radiotherapy alone. However, this impaired outcome in anemic patients is not longer observed when carbogen and nicotinamide are added to radiotherapy, supporting the results of a previous phase II ARCON-trial in head and neck cancer (8).

Attempts have been made to improve outcome of anemic cancer patients using erythropoietin or red blood cell transfusions. A Cochrane review based on five randomized controlled trials found strong indications that for head and neck cancer patients, the addition of erythropoietin to radiotherapy negatively affects patient outcome in terms of loco-regional progression free survival and overall survival (7). Suggested explanations for the lack of benefit of erythropoietin include presence of erythropoietin receptors on the tumor cell membranes stimulating tumor growth, and a decrease of tissue oxygenation due to increased viscosity when Hb concentrations become too high (15,16,17). Head and neck cancer patients with low Hb-levels treated in the DAHANCA 5 (radiotherapy and nimorazole vs radiotherapy and placebo) and DAHANCA 7 (conventional radiotherapy vs accelerated radiotherapy) studies were sub-randomized to receive red blood cell transfusions prior to and during radiotherapy (3). Also with this approach, the increased Hb-level was not able to improve tumor control or survival. Stimulation of inflammatory and immunosuppressive pathways was proposed as a possible factor involved (18). Randomized trials exploring the role of chemotherapy in addition to radiotherapy for patients presenting with anemia are lacking. However, prospective and retrospective studies indicate that the poor prognosis of patients presenting with anemia cannot be improved by a chemoradiotherapy approach (19,20).

The “reduced cord radius” model described by Hirst and colleagues could explain the success of ARCON in anemic patients (21). The term “tumor cord” describes the functional unit of a blood vessel and its dependent tumor cell volume[1*]. The model assumes that the ability of cancer cells to survive at a distance from blood vessels is dependent on the local supply and diffusion distance of oxygen and nutrients from each vessel. Histological examination of tumors in animals exposed to low oxygen tension for several days has shown that the thickness of the tumor cords is less than

1 *Although the typical corded structure is histologically recognizable in some tumors and tumor types, the cord radius model has a broader application. “Cord radius” is the equivalence of the more generally applicable and easier to recognize “distance from blood vessel to necrosis”. However, for readability we use the term “tumor cord” here.

in animals breathing normal air (22). It has been proposed that anemia causes a reduced cord radius by the same mechanism. Restoration of Hb-levels by blood transfusion in anemic animals produced a markedly increased tumor radiosensitivity supposedly by improved oxygenation of tumor cells in the peripheral zones of the cords. However, this effect was only transient and was lost within 24 hours (23). Tumor cords that are chronically exposed to higher oxygen levels, will adapt and begin to proliferate more actively and will once again outgrow their oxygen supply. In contrast, daily carbogen breathing immediately followed by radiotherapy will not cause this adaptive response because the oxygenation increase is too short and only for the duration of the radiation treatment (10-15 min). The status quo of reduced cord radius and, consequently, shorter oxygen diffusion distances in combination with higher levels of free oxygen in plasma explains how ARCON can exploit adaptive mechanisms in anemic patients. Additionally, since the effect of carbogen relies on oxygen transportation by the plasma, the reduced blood viscosity and consequential increased flow through the tumor microvasculature will further benefit the anemic patients. Finally, other compensatory mechanisms such as a shift in the oxygen-hemoglobin dissociation relationship may contribute as well.

Although the effect of hyperbaric oxygen in patients with head and neck cancer has recently been demonstrated in a systematic review and meta-analysis, prospective data testing hyperbaric oxygen in relation to anemia are lacking (24). However, a retrospective analysis of patients with carcinoma of the uterine cervix treated with radiotherapy in hyperbaric oxygen also revealed a marked improvement in local tumor control in patients who were severely anemic prior to radiotherapy (25). Additional support comes from a preclinical study demonstrating that hyperbaric oxygen was successful in overcoming the increased radioresistance associated with anemia in mouse mammary adenocarcinomas (26). These observations are of interest because several mechanisms of action of ARCON discussed above also apply to hyperbaric oxygen.

Another issue is why anemic patients have an inferior outcome compared to non-anemic patients in the first place. It is generally assumed that this is primarily a consequence of the impaired tumor oxygenation resulting in a more aggressive and treatment resistant tumor phenotype (15,27). Indeed, clinical and pre-clinical studies do provide evidence for a correlation between Hb-level and tumor hypoxia measured by polarographic pO_2 electrodes (15,16,28,29). In particular, this association is present in severely anemic patients (Hb <11.0 g/dl (< 6.9 mmol/L)) whereas it is much weaker in patients with mild anemia or low normal Hb-levels. One study did not demonstrate such correlation, but it should be noted that in that study there were hardly any patients with severe anemia (30). This was also the case in the current

study: in the subgroup of patients, participating in the hypoxia marker side study, no correlation was found between tumor hypoxia as measured by pimonidazole binding and Hb-level but there was only one patient with a Hb-level below 11.0 g/dl. Only 79/345 patients took part in this side study. This is mainly due to a late amendment to the initial protocol and the limited number of participating centers to the side study. On the other hand, we argue that it is not the absolute amount of hypoxia but the distribution as function of distance to the vessels that is relevant for the response to ARCON.

Tumor hypoxia may be one reason for the poor prognosis of anemic patients but most likely not the only one. A typical molecular feature of malignancies is the switch in tumor cell metabolism from oxidative to glycolytic pathways (31,32). Anaerobic glycolysis provides cells with a growth advantage in the tumor microenvironment and promotes metastasis formation. With decreased blood viscosity and increased plasma flow in anemic patients there will be greater availability of glucose, fuelling the process of malignant progression. This can explain the observation of an association between anemia and the trend towards a higher N-stage ($P=0.06$) in this study. Apart from a causative factor, anemia can also be an epiphenomenon of aggressive tumor behaviour. Activation of the immune and inflammatory system by the malignant disease produces cytokines, including interferons, tumor necrosis factor and interleukin-1 (33). These cytokines inhibit erythropoiesis, affect the life span of erythrocytes and impair iron metabolism.

Despite reduction of tumor recurrence, no benefit of ARCON was observed on OS in anemic patients. The correlation between low Hb-levels and a poorer performance status ($P<0.01$), observed in this study, suggests that associated co-morbidity in patients with low Hb-levels at diagnosis may affect survival independent of tumor control. Supportive evidence for this comes from the GORTEC 94-01 trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. This study showed that anemia was associated with a higher probability of death caused by intercurrent disease (20). In our cohort of patients presenting with anemia, approximately 40% exhibit significant co-morbidity. It is obvious that the survival impact of co-morbidity cannot be influenced by ARCON or any other cancer treatment (34).

In conclusion, the poor prognosis of laryngeal cancer patients with pre-treatment anemia is not longer observed when radiotherapy is combined with carbogen breathing and nicotinamide. This observation of improved outcome in anemic patients supports an earlier proposed hypothesis (8). Reduced oxygen diffusion distances in the tumor and improved oxygen transportation by the plasma due to reduced blood viscosity

can explain the effectiveness of ARCON in anemic patients. The potential of ARCON should be further explored in a large prospective randomized trial with focus on patients presenting with anemia.

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5

Computed Tomography-based tumour volume as a predictor of outcome in laryngeal cancer: results of the phase 3 ARCON trial

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Abstract

Purpose

Retrospective studies indicate that larger tumour volume is a strong prognostic indicator for poor tumour control after (chemo)radiotherapy for laryngeal cancer. The impact of tumour volume on the outcome of patients treated within a prospective study comparing accelerated radiotherapy (AR) ± carbogen breathing and nicotinamide (ARCON) was investigated.

Methods and Materials

Of 345 patients with cT2-4 laryngeal cancer, pre-treatment CT-scans of 270 patients were available for tumour volume calculation. Contouring of the primary tumour and involved lymph nodes was reviewed by one experienced head and neck radiation oncologist. Kaplan-Meier plots were used for analysis of outcome.

Results

Of 137 AR and 133 ARCON patients, 57 and 80 vs. 56 and 77 patients had glottic and supraglottic tumours, respectively. A correlation between primary tumour volume and T-stage was observed (Rs=.51, $P<.01$). In both treatment arms no correlation was detected between the primary tumour volume and local control (LC), regional control (RC) and metastasis-free survival (MFS). A strong correlation between total nodal volume and N-stage was found (Rs=.93, $P<.01$). Both in the AR and ARCON groups total nodal volume was not associated with poorer RC rate. However, based on individual lymph node analyses, nodal control was in favour of ARCON, irrespective of volume ($P<.01$).

Conclusion

Neither primary tumour volume, nor total nodal volume are prognostic factors for patients with cT2-4 laryngeal cancer treated with accelerated radiotherapy ± carbogen breathing and nicotinamide. Additional analyses based on individual nodal volumes demonstrate an excellent regional control rate and a significant benefit of ARCON.

Introduction

Several retrospective studies demonstrate that the macroscopic primary tumour volume, as calculated from pre-treatment CT- or MR scans, can predict local control in squamous cell carcinoma arising in different subsites of the head and neck area in patients who are treated with definitive (chemo)radiotherapy.[1-4] Threshold volumes have been identified in naso-, oro-, hypopharyngeal and laryngeal tumours to counsel patients regarding the relative likelihood of local tumour control.[1,2] The University of Florida repeatedly demonstrated that volumes greater than 3.5 cm^3 and 6.0 cm^3, respectively for glottic and supraglottic tumours, are inversely related to local control.[5-9] Studer et al. also demonstrated in a cohort of patients with head and neck cancer treated with IMRT that there is a stronger association between primary tumour volume and local control than the association between T-stage and local control.[2]

Less data are available with respect to the volume of involved lymph nodes and nodal control in head and neck cancer. Disease control of involved lymph nodes by radiotherapy decreases with increasing maximum lymph node diameter.[10-12] Only a limited number of reports analysed 3D-CT lymph node volumes and found that total nodal volume was a significant prognostic factor for regional control.[13-16]

In the Netherlands a randomised trial was performed in patients with advanced carcinoma of the larynx comparing accelerated radiotherapy (AR) with accelerated radiotherapy with carbogen breathing and nicotinamide (ARCON) for hypoxic sensitisation. The inhalation of carbogen (98% oxygen + 2% carbon dioxide) decreases diffusion-limited hypoxia and the administration of nicotinamide, a vasoactive agent, reduces perfusion-limited hypoxia. It was demonstrated that hypoxia modulation by ARCON resulted in an improvement of 8% in regional control but there was no effect on local control.[17] Interestingly, in both treatment arms, T-stage was not prognostic for outcome. The purpose of the current study is to analyse the impact of primary tumour volume and nodal volume on prognosis in this large and homogeneous cohort of patients.

Patients and methods

Phase III ARCON trial

This was an open-label, randomised phase III trial comparing AR with ARCON in patients with cT2-4 laryngeal cancer. The trial (ClinicalTrials.gov NCT00147732) was conducted under the auspices of the Dutch Head and Neck Cancer Group in 7 centers in the Netherlands and the UK. Eligibility criteria and details of the procedure

are described previously.[18] Briefly, a radiation dose of 44 Gy in 22 daily fractions of 2 Gy was prescribed to the primary tumour and neck nodes followed by a boost dose of 24 Gy in twice daily fractions of 2 Gy to the primary tumour and involved lymph nodes. Treatment of the neck nodes was by radiotherapy alone, no planned neck dissections were performed. Because a decrease in the radiation tolerance was observed for cartilage and spinal cord in earlier studies with hypoxic sensitisation, the total dose to the larynx and the spinal cord in the ARCON arm was limited to 64 and 40 Gy, respectively.[19,20] Patients allocated to the ARCON arm received carbogen (98% O_2 + 2% CO_2, 4 minutes before and during radiotherapy) and nicotinamide (60 mg/kg, 1-1.5 hours before fractions) concurrently with radiotherapy.[21] Follow-up visits took place every 2, 3, 4 months during the first, second and third year respectively, then every 6 months for another 2 years. The larynx was assessed by fiberoptic or indirect laryngoscopy. Regional control was assessed by palpation of the neck. When tumour recurrence was suspected, imaging (CT-scan or MRI) was performed to document the extent of the disease and biopsies were taken for pathological confirmation.

Approval for the study was obtained from the Radboud University Nijmegen Medical Centre Research Ethics Committee with ratification from each centre. Written informed consent was obtained before randomisation.

Volumetric analysis

All pre-treatment CT-scans were evaluated by a head and neck radiologist. Contouring of the primary tumour and involved lymph nodes was reviewed by one experienced head and neck radiation oncologist. The majority of the imaging studies were performed during intravenous injection of contrast medium. A slice thickness of ≤ 3 mm was used in most cases. For every CT-study, the outer margin of any abnormal laryngeal mass or involved lymph node was contoured on each image. Neck nodes were considered pathological in case of the presence of central necrosis on CT-scan and/or positive cytology and in case of a minimal axial diameter on CT-scan of more than 10 mm. Indications for a cytological punction were the presence of a lymph node with a short axis diameter ≥5 mm, the loss of a nodal hilum, a nodular shape or the presence of necrosis. Location of the initially involved and recurrent nodes to specific lymph node levels was assessed according to the consensus recommendations for delineation of node levels by Gregoire et al.[22] The tumour volume was calculated automatically in cubic centimetres by multiplying the tumour areas by section thickness and intersection gap (summation-of-areas technique).

Endpoints

Endpoints were local control (LC), regional control (RC) and metastasis-free survival (MFS) at 5 years from randomisation. All intervals were calculated from the date of randomisation and censored after 60 months or at last follow-up. LC was defined as complete and persistent disappearance of the primary tumour after radiotherapy, and as freedom of first recurrence in the larynx, not including salvage procedures. RC was defined as complete and persistent disappearance of the pathological lymph node(s), and freedom of first recurrence in the lymph nodes after radiotherapy, not including salvage procedures. MFS was defined as the time from randomisation to distant metastasis. In addition, an analysis per individual lymph node was performed, which is referred to as nodal control (NC).

Statistical Methods

Statistical analyses were performed using SPSS 19.0 for Mac. Mann-Whitney U and Chi-square tests, where appropriate, were used at a two-sided significance level of .05. Kaplan-Meier plots with log-rank testing and Cox proportional hazard models were used to investigate the impact of volume on radiotherapy outcome. Tumour volume was analysed as a continuous variable, by receiver operating characteristic (ROC analysis) and by dichotomisation at previously described cut-off values of 3.5 cm^3 for glottic and 6.0 cm^3 for supraglottic tumours, and by the median for nodal volume.[5,8]

Role of the funding source

The study sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Patient Characteristics

Between April 2001 and February 2008, 345 patients were randomised to either AR (n=174) or ARCON (n=171). Pre-treatment CT-scans of 270 patients (AR, n=137; ARCON, n=133) were available for tumour volume calculation. For technical reasons, pre-treatment CT-scans could not be used or restored in the remaining 75 patients. The median follow-up time was 44 months (range 2-84) and 59 (range18-84) months for the whole group and for patients still alive, respectively. Median age was 60 years for AR and ARCON patients. Male/female ratio was 109/28 and 108/25 for AR and ARCON, respectively. Tumour characteristics were well balanced without significant differences between the treatment arms (Table 1).

Table 1 Tumour characteristics.

Characteristics	AR (N=137)	ARCON (N=133)	*P* [a]
Site of the primary tumour - no. (%)			.49
Glottic	57 (42)	56 (42)	
Supraglottic	80 (58)	77 (58)	
AJCC stage groupings - no. (%)			.85
Stage II	41 (30)	36 (27)	
Stage III	56 (41)	59 (44)	
Stage IV	40 (29)	38 (29)	
T-stage - no. (%)			.79
T2	50 (37)	43 (32)	
T3	69 (50)	74 (56)	
T4	18 (13)	16 (12)	
N-stage - no. (%)			.89
N0	93 (68)	90 (68)	
N1	18 (13)	16 (12)	
N2a	2 (1)	2 (1)	
N2b	8 (6)	6 (5)	
N2c	16 (12)	19 (14)	
N3	0 (0)	0 (0)	
Primary tumour volume in cm^3 - range (median)			.79
Glottic	.3 - 34.8 (3.6)	.3 - 49.9 (3.0)	
Supraglottic	2.1 - 37.5 (11.1)	.8 - 42.7 (10.8)	
Total nodal volume in cm^3 - range (median)	.4 - 57.7 (3.6)	1.0 - 65.2 (3.5)	.80

[a] Chi-Square test

Primary tumour volume

A correlation between the primary tumour volume and T-stage was observed (Rs=.51, $P < .01$). In both treatment arms no correlation was found between the primary tumour volume and local control (LC), regional control (RC) or metastasis-free survival (MFS) when a cut-off value of 3.5 cm^3 for glottic and 6.0 cm^3 for supraglottic tumours was used (Figure 1). Similar observations were done when volume was analysed as a continuous variable or by ROC analysis (data not shown). In an exploratory subgroup

analysis we also did not find a correlation between primary tumour volume and local control rate when stratified for T-stage.

Lymph node volume

Eighty-seven patients had pathological lymph nodes and the total number of involved lymph nodes in these patients was 165.

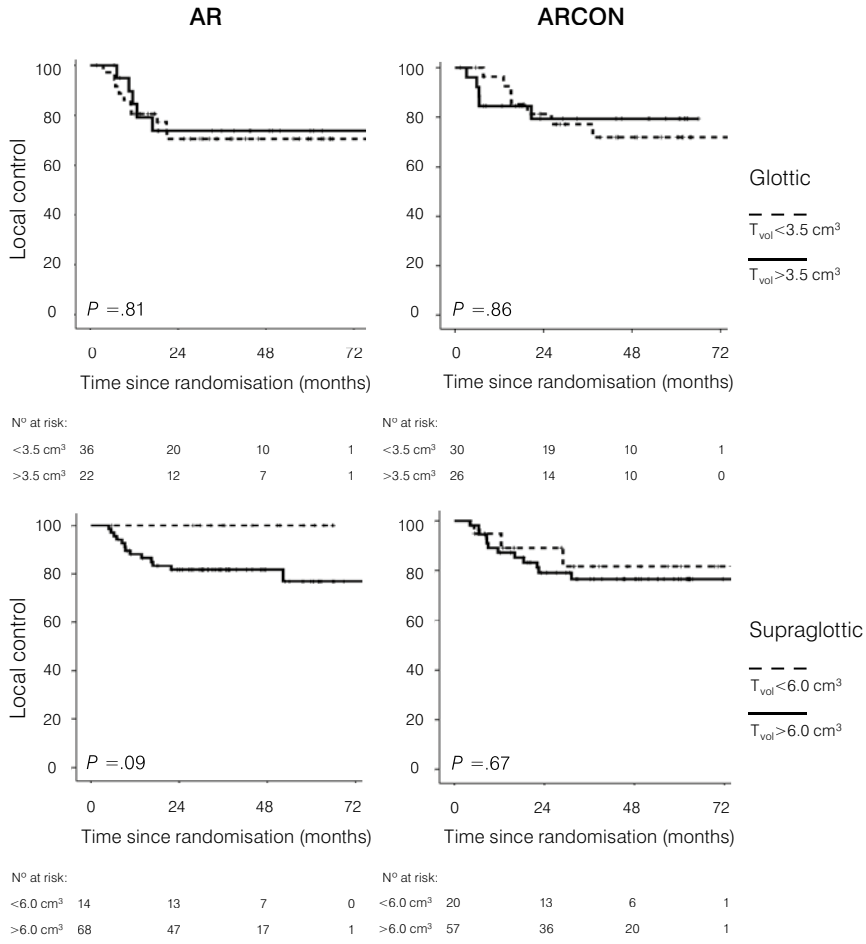

Figure 1 No correlation is observed between the primary tumour volume at glottic or supraglottic subsite and the local control for patients treated by AR or ARCON.

A strong correlation between total nodal volume and N-stage was found (Rs=.93, P <.01). The total nodal cut-off value of 3.5 cm^3 in the current study, which corresponds to a sphere with a diameter of 2.0 cm, is based on the median value of the total nodal volumes. The presence of pathological lymph nodes (N0 vs. N+) was a poor prognostic factor for RC (Figure 2). However, in the AR and ARCON groups total nodal volume (< 3.5 cm^3 vs. > 3.5 cm^3) was not associated with a poorer RC. MFS of patients with neck level IV involvement was worse compared to other patients, independent of treatment regimen (P <.01).

When an analysis based on individual lymph nodes was performed, an inferior 5 year nodal control rate was observed in both treatment arms for larger nodal volumes with a cut-off value of 3.5 cm^3 (AR: 79% vs. 54%, P <.01 and ARCON: 98% vs. 80%, P .02). Regional control was in favour of ARCON for nodal volumes < 3.5 cm^3 (AR: 79% vs. ARCON: 98%, P <.01) and volumes > 3.5 cm^3 (AR: 54% vs. ARCON: 80%, P <.01) (Figure 3).

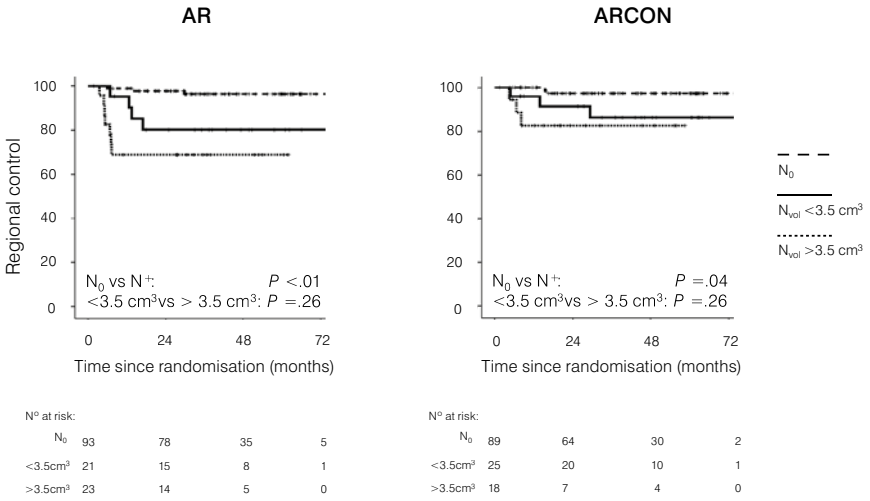

Figure 2 The presence of pathological lymph nodes is a poor prognostic factor for regional control, while larger total nodal volume was not associated with a poorer regional control.

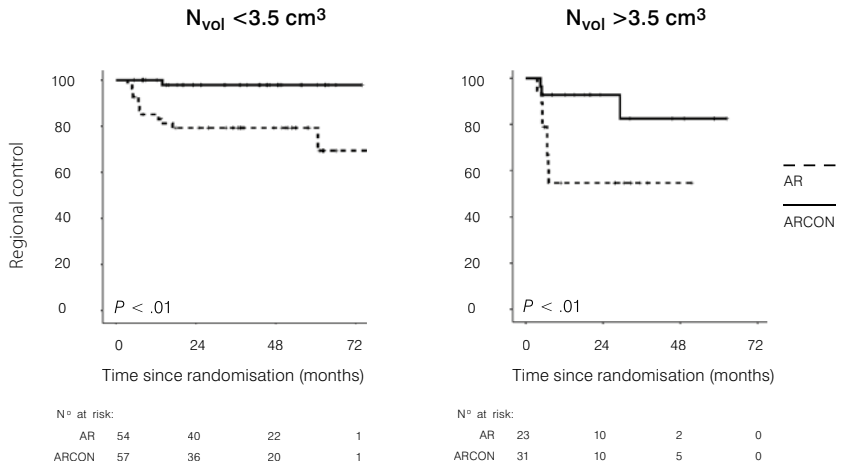

Figure 3 Based on individual lymph node analyses, nodal control was in favour of ARCON, irrespective of nodal volume.

5

Discussion

The strong prognostic value of primary tumour volume, observed in several retrospective analyses was not confirmed in patients with cT2-4 laryngeal cancer treated in a large prospective randomised trial with accelerated radiotherapy with or without carbogen breathing and nicotinamide.[3-9]

Repeated analyses reported by the University of Florida demonstrated that pre-treatment based CT-volumes smaller than 3.5 cm^3 for glottic and 6.0 cm^3 for supraglottic tumours resulted in a significant better local control.[5-9] One possible explanation for the absence of a volume effect in the current study might be the reduction of overall treatment time by the use of accelerated radiotherapy for all patients. This approach has demonstrated superior loco-regional tumour control rates in several head and neck cancer trials.[23-25] With conventional fractionation schedules local control rates are in the order of 67-80%, 30-77% and 26-52% for T2, T3 and T4 tumours, respectively.[26-30] A previous analysis of the current study failed to show a correlation between local tumour control and T-stage, even when glottic and supraglottic tumors were analysed separately.[17] These observations indicate that patients with more advanced T-stages and larger tumour volumes profit most from accelerated fractionation. Evidence for this has recently been published by Soliman

et al. for a large cohort of lung cancer patients from the CHARTWEL trial (Continuous Hyperfractionated-Accelerated RadioTherapy WEekend-Less) treated in one single institution.[31] In this randomised trial, the conventional dose of 66 Gy in 33 daily fractions of 2 Gy over 6.5 weeks was compared to 60 Gy in 40 fractions of 1.5 Gy, 3 fractions per day, over 18 days. CHARTWEL was increasingly more efficient relative to conventional fractionation with increasing tumour volume. The authors speculated that larger tumours may have a higher capacity to repopulate during radiotherapy, e.g. because they might be more hypoxic and more cancer stem cells might start rapid repopulation after radiation induced reoxygenation. Another important reason for the lack of correlation between tumour volume and local control can be explained by methodology. The current analysis is based on a large cohort of tumours treated in a homogenous way by 3D-conformal radiotherapy in thermoplastic masks and position verification. Although tumour volume calculations in the retrospective studies are based on diagnostic CT-or MR imaging, the radiotherapy treatment planning was performed based on conventional 2D equipment.[3-9] Despite the use of accelerated or hyperfractioned radiotherapy regimens, it might be that larger tumours in particular are subjective to local failures by underestimating tumour extension due to the 2D-treatment planning and lack of position verification systems.[3-9]

It has also been demonstrated by a limited number of reports that increasing nodal volume, based on the total volume of all neck nodes, is associated with poor RC.[13-16] Although the presence of pathological lymph nodes in the current study was a poor prognostic factor for regional control, no correlation between total nodal volume and RC was observed in both treatment arms. This could be explained by the high regional control rates and the limited number of events. To increase the study size and number of events, an additional analysis per lymph node was performed. This time, an inferior 5 year nodal control rate was observed in both treatment arms for increasing nodal volumes. The additional analysis also demonstrated that ARCON improved nodal control for nodal volumes up to 3.5 cm^3 and larger compared to AR. This observation in a well-balanced group of glottic and supraglottic patients with pathologic neck nodes, receiving similar doses of radiotherapy in both treatment arms, supports the role of hypoxia in the occurrence of regional failure. In the DAHANCA 6&7 trial, a benefit of regional control was not observed by increasing the number of fractions per week from 5 to 6, suggesting a minor contribution of repopulation in the neck nodes.[24] Our study is the first reported prospective randomised trial employing a treatment that is able to correct the poor outcome of increasing nodal volume in a homogenous cohort of head and neck cancer patients. Nodal volume calculation remains difficult to compare with literature for multiple reasons (32). Reports are retrospective and combine different head and neck subsites with a different radiosensitivity such as HPV or EBV positive and negative

tumours.[33,34] Reports also use a variety of treatment strategies and methods for measuring volumes (sphere and ellipsoid formula, summation-of-areas technique). Moreover, nodal volumes, as a separate entity, are reported only in a minority of papers using different endpoints (loco-regional control, regional control, metastasis-free survival, overall survival). Although the maximal diameter of regional lymph nodes is far from an accurate measure of tumour load in the neck, today it remains the standard for decision making on additional neck dissection or chemo-radiotherapy in daily practice.[10-12]

Conclusion

The strong prognostic value of primary tumour volume, observed in a number of retrospective analyses was not confirmed in patients treated in this prospective randomised trial with 3D-conformal, accelerated radiotherapy ± carbogen breathing and nicotinamide. In patients with pathological lymph nodes, a correlation between total nodal volume and regional control was lacking as well. Additional analyses based on individual nodal volumes demonstrated an inferior nodal control with increasing nodal volume for both regimens. However, a significant benefit in nodal control was observed in favour of ARCON.

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6

Quality-of-Life after radiotherapy for advanced laryngeal cancer: results of a phase III trial of the Dutch Head and Neck Society

Submitted

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Abstract

Background

To report on health-related quality-of-life (HRQoL) of 345 patients with cT2-4 laryngeal cancer, treated in a randomized trial comparing accelerated radiotherapy with carbogen and nicotinamide (ARCON) against accelerated radiotherapy alone (AR).

Methods

HRQoL was assessed using the European Organisation for Research and Treatment of Cancer (EORTC) HRQoL Questionnaire-C30 (QLQ-C30) and the Head & Neck cancer module (QLQ-H&N35) at baseline, at completion of radiotherapy and at 6, 12, and 24 months post-baseline. Data were analyzed two years after inclusion of the last patient.

Results

Local tumor control at 5 years was 78% for AR versus 79% for ARCON. Moderate to severe clinical impact of the treatment was observed for nearly all items of the QLQ-C30 and QLQ-H&N35 between baseline and end of treatment. At 6 months, scores returned to baseline level with exception of dry mouth, sticky saliva, and taste/smell. No difference in HRQoL score between AR and ARCON was observed. At 2 years from baseline, the percentage of patients reporting moderate to severe complaints of dry mouth, sticky saliva, or changes in taste/smell was 30%, 22% and 18%, respectively, while the majority of patients had no or few complaints of swallowing (79%) or speech (64%). The use of a feeding tube at 2 years from diagnosis was limited to 5% of patients.

Conclusions

With accelerated radiotherapy, high local tumor control was obtained while maintaining good speech and swallowing function. Long-term dry mouth, sticky saliva and changes in taste/smell are limited to one quarter of patients.

Introduction

Treatment of patients with head and neck cancer has a significant impact on health-related quality-of-life (HRQoL). A substantial percentage of survivors will suffer from long-term disease specific side effects (e.g. xerostomia, sticky saliva and dysphagia) and general adverse symptoms like fatigue, difficulties in social functioning and mental problems (1,2,3,4,5,6). In head and neck cancer, there has been a shift from surgery towards organ preservation strategies over the last decades. These strategies comprise of radiotherapy with altered fractionation schedules and concomitant chemoradiotherapy, sometimes even combined with neoadjuvant chemotherapy (7,8). The price for these intensified regimens is increased acute and long-term toxicity (9,10). A balanced decision-making between increased toxicity and modest improvements in tumor control is often difficult for patients and treating physicians. Thus, proper knowledge of HRQoL is essential to understand the real benefit of any new regimen. For this reason questionnaires on HRQoL have been fully integrated in the majority of phase III trials (11). The European Organisation for Research and Treatment of Cancer (EORTC) Questionnaire-C30 (QLQ-C30) and the Head&Neck cancer module (QLQ-H&N35) are amongst the most commonly used questionnaires (12,13,14). Although an increasing number of studies on HRQoL from clinical trials in head and neck cancer is being published, prospectively collected long-term data on large and homogeneous populations like patients with advanced laryngeal cancer are still sparse (1,3,4,15,16).

Recently it was demonstrated in a randomized phase III trial that the addition of carbogen breathing and nicotinamide to accelerated radiotherapy (ARCON) in patients with T2-4 laryngeal cancer results in a 87% larynx preservation rate and a benefit of 8% in regional control compared to accelerated radiotherapy (AR) alone (17). In subgroups (hypoxic tumors, anemic patients) the gain was significantly larger (17,18). Accelerated radiotherapy counteracts tumor cell repopulation whereas carbogen breathing and nicotinamide reduce hypoxic radioresistance (19). There was a good compliance rate with carbogen breathing (86%) and nicotinamide (80%) and similar acute and late toxicity profiles were observed between the arms of the study (20). Information on HRQoL can help to further determine the real therapeutic benefit of ARCON and may assist in decision-making regarding larynx preservation treatments for patients with advanced laryngeal cancer.

This paper reports on the impact of AR and ARCON on HRQoL in patients presenting with locally advanced laryngeal cancer.

Methods

Study Design and Eligibility

This was an open-label, randomized phase III trial comparing AR with ARCON in patients with cT2-4 laryngeal cancer. The trial (ClinicalTrials.gov NCT00147732) was conducted under the auspices of the Dutch Head and Neck Society in 7 centers in the Netherlands and the UK (Table S1). Eligibility criteria are provided in the supplementary Table S2.

Approval for the study was obtained from the Radboud University Medical Centre Research Ethics Committee Nijmegen with ratification from each center. Written informed consent was obtained before randomization.

Randomization

Patients were centrally randomized by phone at the IKO ("Integraal Kankercentrum Oost") trials office. Treatment arm assignments (AR vs ARCON) were stratified for tumor site (glottic vs supraglottic) and institution. A dynamic allocation method was used to avoid imbalance of treatment assignment within an institution. Randomization took place after completion of all study investigations and no longer than 4 weeks prior to the anticipated start of treatment.

Treatment

A radiation dose of 44 Gy in 22 daily fractions of 2 Gy was prescribed to the primary tumor and regional neck nodes followed by a boost dose of 24 Gy in twice daily fractions of 2 Gy to the primary tumor and involved lymph nodes. Details on target volume and technique are described earlier (17,20). Because a decrease in the radiation tolerance was observed for cartilage and spinal cord in earlier studies with hypoxic sensitization, the total dose to the arytenoid cartilage and the spinal cord in the ARCON arm was limited to 64 and 40 Gy, respectively (21,22).

Patients allocated to the ARCON arm received carbogen (98% O_2 + 2% CO_2, 4 minutes before and during radiotherapy) and nicotinamide (60 mg/kg, 1-1.5 hours before fractions) concurrently with radiotherapy. Details of the procedure are described previously (17,20).

All patients participating in this study were treated in academic centers with accreditation for head and neck oncology by the Dutch Head and Neck Society and employing institution-wide quality assurance programs.

Health Related Quality-of-Life

The validated EORTC quality-of-life core measure Questionnaire-C30 (QLQ-C30, version 3) and the disease-specific Head & Neck cancer module (QLQ-H&N35) were used (12,13,14). The QLQ-C30 comprises 5 functional scales (physical, cognitive, emotional, social and role), a global quality-of-life scale, 3 symptom scales (pain, fatigue and nausea/vomiting) and 6 single items (dyspnea, insomnia, appetite loss, constipation, diarrhea and financial difficulties). The QLQ-H&N35 is composed of 7 symptom scales (pain, swallowing, senses, speech, social eating, social contact and sexuality), 6 symptom items (teeth, trismus, dry mouth, sticky saliva, cough and feeling ill) and 5 additional items concerning the use of pain medication, nutritional supplements, feeding tube and weight loss or weight gain.

Questions on both HRQoL measures were scaled and scored using the recommended EORTC Quality-of-Life Group procedures (23). Raw scores were transformed to a linear scale ranging from 0 to 100. A higher score represents a higher level of functioning or a higher level of symptoms. Evidence-based guidelines were developed for QLQ-C30 to classify differences from trivial to large (24). For the interpretation of the QLQ-H&N35 questionnaires, mean changes of <10 effect-points were considered as no or small changes in QoL. Mean changes of >20 points were classed as large effects in QoL (25).

Monitoring During Treatment and Follow-up Evaluations

At baseline, at completion of radiotherapy and at 6, 12, and 24 months post-baseline, quality-of-life was assessed using the QLQ-C30 and the QLQ-H&N35 questionnaires. Follow-up visits took place every 2, 3, 4 months during the first, second and third year respectively, then every 6 months for another 2 years. The larynx was assessed by fiberoptic or indirect laryngoscopy. Regional control was assessed by palpation of the neck. When tumor recurrence was suspected, imaging (CT-scan or MRI) was performed to document the extent of the disease and biopsies were taken for pathological confirmation. HRQoL assessments after salvage procedures were not included in this analysis.

Endpoints and Statistics

The primary endpoint of the randomized trial was local control. Intervals were calculated from the date of randomization and censored after 60 months or at last follow-up. Local Control (LC) and regional control (RC) were defined as freedom of first recurrence at the primary tumor site or neck and complete and persistent disappearance of the pathological lymph nodes after radiotherapy, not including salvage procedures. Compliance levels on QoL were calculated based on the number of questionnaires received divided by the number of questionnaires expected at each

assessment point. For every patient without tumor-related event, in follow-up with QoL assessment at baseline, a questionnaire was expected. Patients were evaluable for QoL assessment if at least the baseline questionnaire and one additional questionnaire, prior to disease recurrence, were available.

Statistical analyses were performed using SPSS 19.0.0 for Mac. Changes in symptoms and QoL items were evaluated with a repeated measures ANOVA using a mixed effect modeling procedure. F-tests were used for testing main effects of group and time, and an interaction effect of group x time. For comparison of continuous variables between the study arms the Mann-Whitney test was used. Differences in compliance between arms was tested using the Fisher's exact test. The level of statistical significance was set at $P = .05$.

Role of the funding source

The study sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

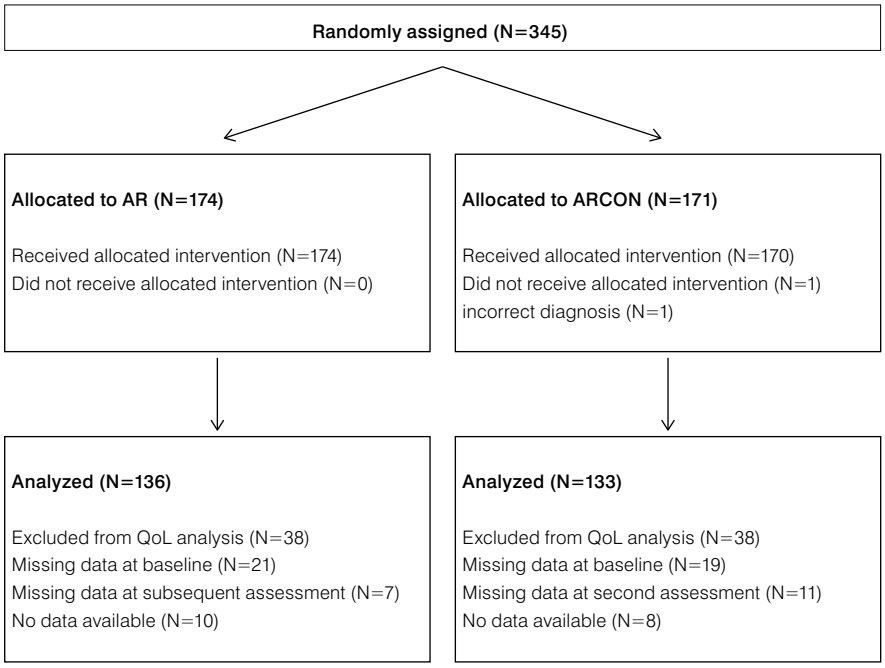
Results

Patient Characteristics and Tumor-related Outcome

Between April 2001 and February 2008, 345 patients were randomized to either AR (n=174) or ARCON (n=171) (Figure 1). Patient demographics and clinical tumor characteristics were well balanced without significant differences between the treatment arms (Table 1). After a median follow-up of 44 months, local tumor control rate at 5 years was 78% for AR versus 79% for ARCON ($P = .80$) with corresponding larynx preservation rates of 84% and 87% ($P = .48$). The 5-year regional control was significantly better with ARCON compared to AR (93% vs. 86%, $P = .04$). AR and ARCON produced equal levels of acute and late toxicity (17,20). Twelve patients in the AR group and 6 patients in the ARCON group received a tracheostomy for severe edema with dyspnea and/or stridor. All 27 (AR: n=15, ARCON: n=12) patients who developed a cartilage necrosis could be managed conservatively. Four of them did receive a tracheostomy (AR: n=1, ARCON: n=3) and there was no need for laryngectomy.

Compliance

The overall compliance with completion of questionnaires was 78% at baseline, 68% at the end of treatment, 70%, 70% and 65% at 6, 12, and 24 months, respectively. Compliance for patients treated by AR or ARCON at different time points is listed in



AR: Accelerated Radiotherapy
ARCON: Accelerated Radiotherapy with Carbogen and Nicotinamide

Figure 1 CONSORT diagram.

Table 2. No significant difference was observed between both treatment arms. HRQoL data on 76 patients were excluded from analysis due to missing data at baseline (N=40), at a subsequent assessment moment (N=18) or at any moment of assessment (N=18). No difference in patient, tumor and treatment characteristics was observed between included and excluded patients (Mann-Whitney or Fisher's exact tests of age, sex, tumor site, T, N, M, stage and treatment arm).

EORTC QLQ-C30, version 3.0

Moderate to large differences were observed between baseline and end-of-treatment measurements in 10 out of 15 items (Figure 2). Large clinical effects were observed for role functioning, global health, fatigue, nausea/vomiting, pain and loss of appetite, all to the worse. Moderate differences were observed for physical-, cognitive-, and social functioning, as well as constipation. No significant differences were observed between patients treated by AR or ARCON. At 6 months post-baseline

Table 1 Patient demographics and clinical tumor characteristics (N=345).

Characteristics	AR (N=174)	ARCON (N=171)	*P*
Age - yr			0.56[a]
Median	60	61	
Range	38-88	41-84	
Sex - no. (%)			0.16[b]
Male	136 (78)	142 (83)	
Female	38 (22)	29 (17)	
Performance status - no. (%)			0.54[b]
0	140 (80)	137 (81)	
1	34 (20)	34 (19)	
Site of the primary tumor - no. (%)			0.49[b]
Supraglottic	100 (57)	97 (56)	
Glottic	74 (43)	74 (44)	
AJCC stage groupings - no. (%)			0.35[b]
Stage II	53 (31)	46 (27)	
Stage III	65 (37)	77 (45)	
Stage IV	56 (32)	48 (28)	
T-stage - no. (%)			0.20[b]
T2	67 (38)	55 (32)	
T3	80 (46)	95 (56)	
T4	27 (16)	21 (12)	
N-stage - no. (%)			0.58[b]
N0	117 (67)	116 (68)	
N1	20 (12)	23 (13)	
N2a	4 (2)	7 (4)	
N2b	10 (6)	5 (3)	
N2c	23 (13)	20 (12)	
N3	0 (0)	0 (0)	

[a] Mann-Whitney U test
[b] Chi-Square test

all items returned to baseline level, except fatigue and cognitive functioning (both small differences compared to baseline). At 24 months insomnia was slightly less compared to baseline score ($P < .01$) (Figure 2K).

Table 2 Compliance with questionnaires at each assessment point for patients treated by AR and ARCON (N=345).

Characteristics	AR (N=174)			ARCON (N=171)			*P* [a]
	Received (N)	Expected (N)	Compliance (%)	Received (N)	Expected (N)	Compliance (%)	
At Baseline	136	174	78	133	171	78	0.76
At completion of radiotherapy	114	173	66	119	169	70	0.37
At 6 months	109	159	69	115	159	72	0.46
At 12 months	93	136	68	99	139	72	0.61
At 24 months	69	115	60	79	113	70	0.12

[a] Fisher's exact test

EORTC QLQ-H&N35

Large clinical impact (> 20 points difference) was observed for the majority of items between baseline and end of treatment (Figure 3) but also for this disease-specific HRQoL scoring no differences between AR and ARCON were observed. At 6 months, scores returned to baseline level for all items with exception of dry mouth, sticky saliva, and taste/smell (Figure 3C, 3J, 3K). For the latter items, further improvement was observed at 12 and 24 months with a return to baseline levels for the majority of patients with dry mouth and sticky saliva. At 2 years, the percent of patients reporting "quite a bit" or "very much" complaints of dry mouth, sticky saliva, or changes in taste- and smell perception was 30%, 22% and 18%, respectively.

Speech and swallowing

Up to 12 months post-baseline, swallowing function improved to baseline level whereas speech continued to improve in the second year and went on to exceed

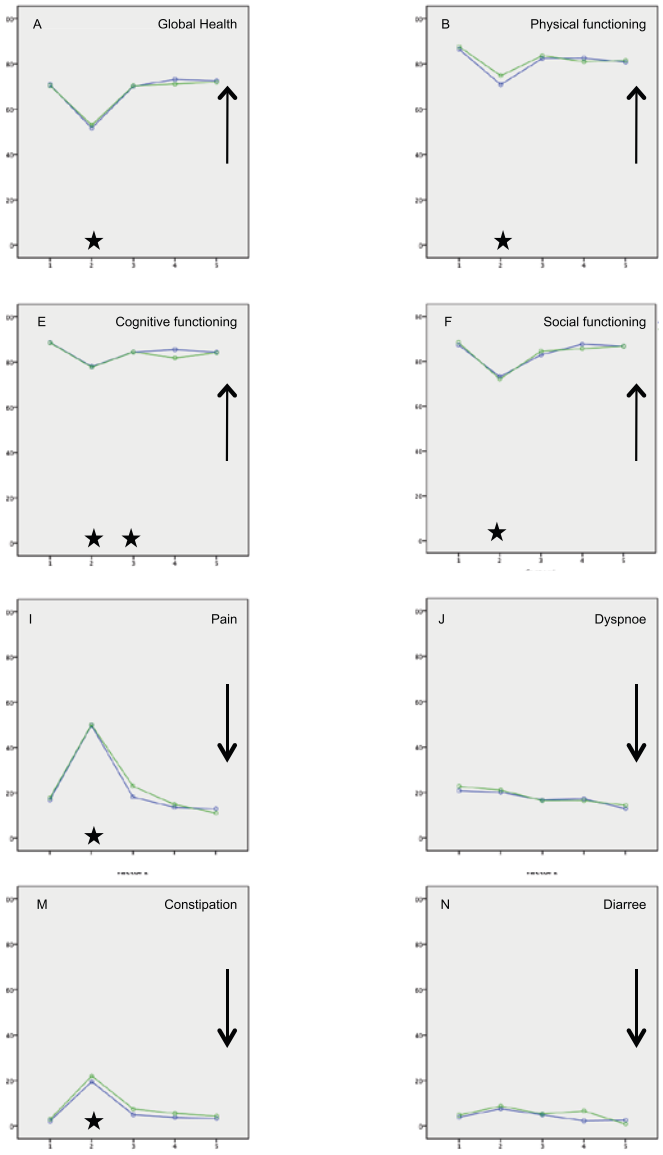


Figure 2 EORTC QLQ-C30, version 3.0, average score (Y-axis) for AR (blue line) and ARCON (green line) patients at baseline, at completion of radiotherapy and at 6, 12, and 24 months post-baseline (X-axis). Moderate to severe changes were observed between baseline and end of treatment measurements in 10 out of 15 items. At 6 months small differences are still observed for cognitive functioning (E) and fatigue (G).

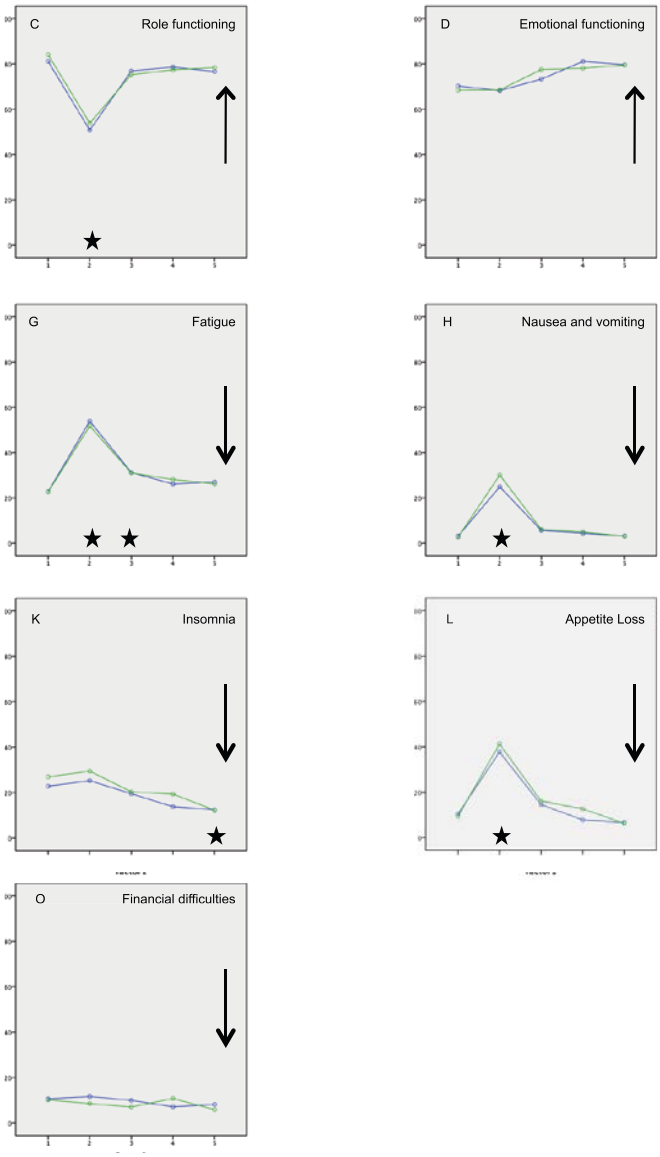


Figure 2 (Continued) At 24 months insomnia (K) was slightly less compared to baseline scores. No significant differences were observed between patients treated by AR or ARCON. Statistically significant changes relative to baseline are indicated by ★, the arrow indicates improvement, i.e. a higher level of functioning or less symptoms.

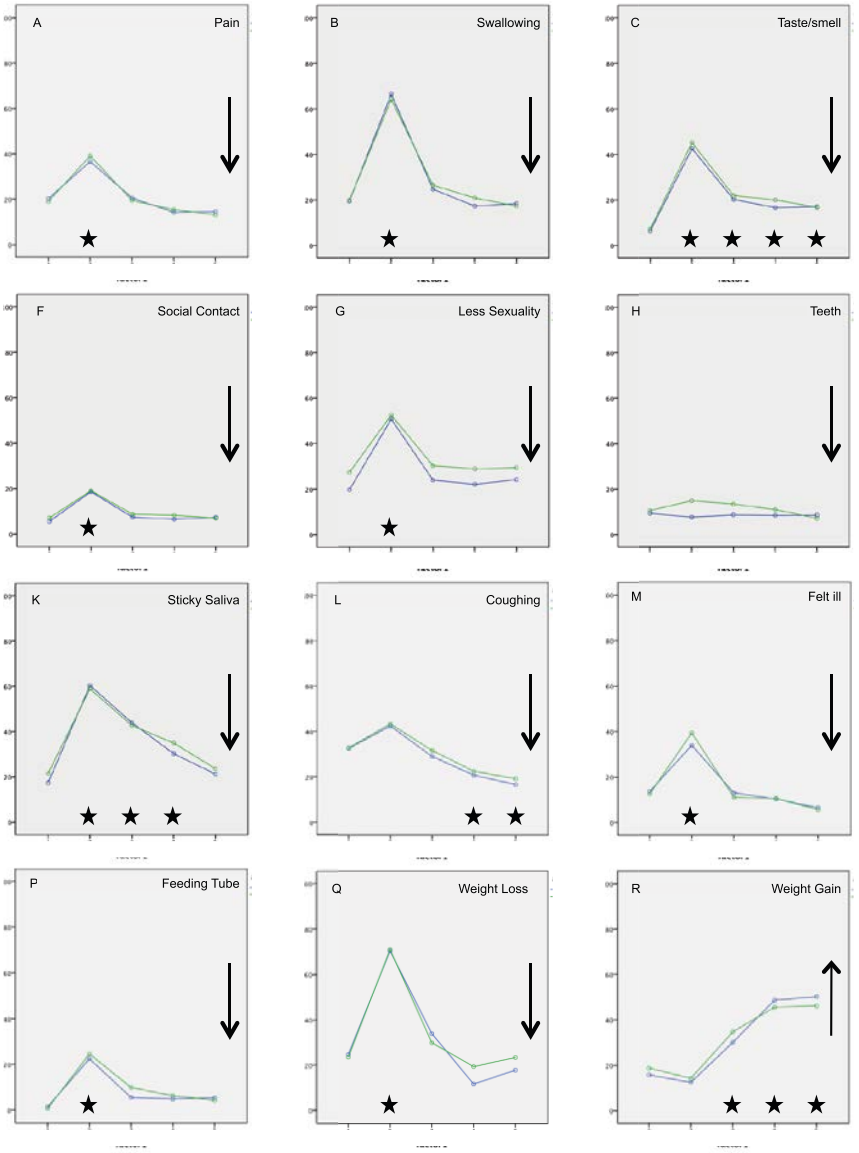


Figure 3 EORTC QLQ-H&N35, average score (Y-axis) for AR (blue line) and ARCON (green line) patients at baseline, at completion of radiotherapy and at 6, 12, and 24 months post-baseline (X-axis). At 6 months, scores returned to baseline level for all items with exception of, taste- and smell perception (C), dry mouth (J), sticky saliva (K).

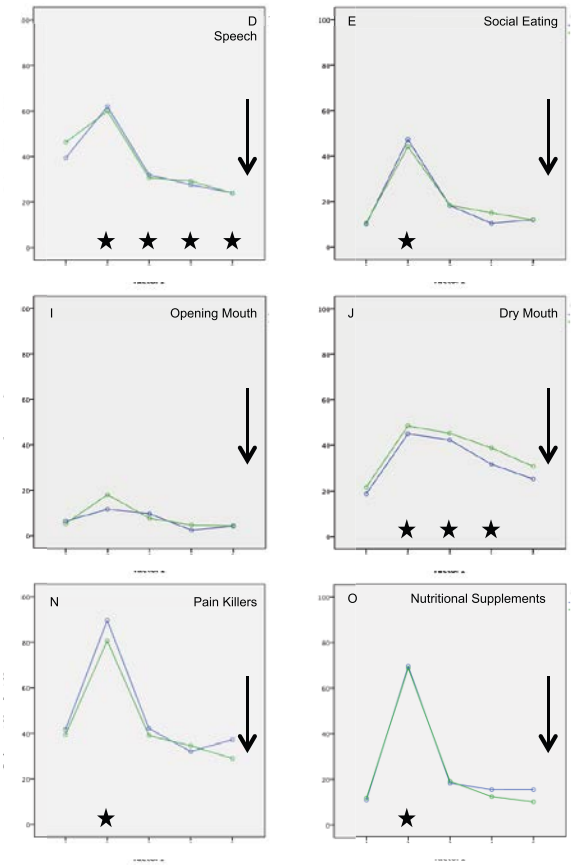


Figure 3 (Continued) Swallowing function (B) improved to baseline level but not further thereafter. Speech (D) continued to improve in the second year and went on to exceed pre-treatment levels. Statistically significant changes relative to baseline are indicated by ★, the arrow indicates improvement, i.e. a higher level of functioning or less symptoms.

Table 3 Tumor- and patient related factors (median) with potential impact on QLQ-H&N35 score for speech and swallowing at different time-points (in bold: P = < .05).

			Speech				
			Baseline	End RT	6m	12m	24m
T-stage	T2-3 vs T4	AR	**33 vs 56**	67 vs 72	22 vs 22	**11 vs 56**	17 vs 22
		ARCON	**44 vs 67**	56 vs 44	22 vs 33	22 vs 33	22 vs 0
		All	**33 vs 56**	67 vs 67	22 vs 33	22 vs 33	22 vs 22
N-stage	N0 vs N+	AR	33 vs 44	67 vs 67	22 vs 22	11 vs 22	22 vs 11
		ARCON	44 vs 44	67 vs 56	22 vs 22	22 vs 33	22 vs 22
		All	44 vs 44	67 vs 56	22 vs 22	22 vs 22	22 vs 19
Site	Glottic vs Supraglottic	AR	44 vs 33	67 vs 67	22 vs 22	11 vs 22	22 vs 19
		ARCON	**56 vs 33**	67 vs 56	22 vs 22	22 vs 33	22 vs 22
		All	**44 vs 33**	67 vs 56	22 vs 22	11 vs 22	22 vs 22
T-volume	Supraglottic (< vs > 6 cm^3)	AR	33 vs 33	67 vs 67	22 vs 22	17 vs 22	22 vs 11
	Glottic (< vs > 3.5 cm^3)	ARCON	44 vs 44	61 vs 56	22 vs 22	22 vs 33	17 vs 22
		All	39 vs 44	67 vs 67	22 vs 22	22 vs 22	22 vs 14
PS	0 vs 1	AR	33 vs 50	**67 vs 78**	22 vs 22	22 vs 28	22 vs 6
		ARCON	44 vs 56	56 vs 61	22 vs 22	22 vs 33	22 vs 22
		All	33 vs 56	**56 vs 72**	22 vs 22	22 vs 33	22 vs 17
Age	<70 vs >70	AR	33 vs 44	**67 vs 78**	22 vs 33	22 vs 22	22 vs 19
		ARCON	44 vs 44	56 vs 61	22 vs 33	22 vs 33	22 vs 33
		All	44 vs 44	56 vs 67	22 vs 33	22 vs 33	22 vs 22
Gender	Male vs Female	AR	33 vs 33	67 vs 67	22 vs 17	11 vs 22	19 vs 22
		ARCON	44 vs 56	**56 vs 78**	22 vs 33	22 vs 33	22 vs 11
		All	44 vs 39	67 vs 67	22 vs 22	22 vs 22	22 vs 17

			Swallowing				
			Baseline	End RT	6m	12m	24m
T-stage	T2-3 vs T4	AR	8 vs 4	75 vs 71	17 vs 25	8 vs 17	8 vs 8
		ARCON	8 vs 25	75 vs 58	17 vs 17	17 vs 25	8 vs 8
		All	8 vs 8	75 vs 67	17 vs 17	8 vs 21	8 vs 8
N-stage	N0 vs N+	AR	**8 vs 25**	75 vs 67	17 vs 25	8 vs 17	8 vs 13
		ARCON	**8 vs 25**	67 vs 75	17 vs 17	17 vs 17	8 vs 8
		All	**8 vs 25**	75 vs 75	17 vs 24	8 vs 17	8 vs 8
Site	Glottic vs Supraglottic	AR	**0 vs 25**	75 vs 75	17 vs 17	8 vs 17	8 vs 8
		ARCON	**0 vs 25**	63 vs 75	17 vs 25	8 vs 17	8 vs 13
		All	**0 vs 25**	67 vs 75	17 vs 22	8 vs 17	8 vs 8
T-volume	Supraglottic (< vs > 6 cm^3)	AR	8 vs 17	75 vs 75	17 vs 25	8 vs 8	8 vs 8
	Glottic (< vs > 3.5 cm^3)	ARCON	**8 vs 25**	**58 vs 75**	17 vs 17	17 vs 17	8 vs 8
		All	**8 vs 17**	67 vs 75	17 vs 19	8 vs 11	8 vs 8
PS	0 vs 1	AR	**8 vs 25**	**67 vs 83**	17 vs 21	8 vs 8	8 vs 0
		ARCON	8 vs 21	75 vs 71	17 vs 17	17 vs 17	8 vs 29
		All	**8 vs 25**	75 vs 75	17 vs 17	8 vs 17	8 vs 8
Age	<70 vs >70	AR	8 vs 8	75 vs 83	**17 vs 50**	**8 vs 21**	8 vs 25
		ARCON	**17 vs 0**	75 vs 67	22 vs 17	17 vs 11	**8 vs25**
		All	8 vs 4	75 vs 75	17 vs 20	8 vs 17	**8 vs 25**
Gender	Male vs Female	AR	**8 vs 25**	67 vs 75	17 vs 17	8 vs 8	8 vs 8
		ARCON	8 vs 25	**67 vs 83**	17 vs 29	10 vs 25	8 vs 22
		All	**8 vs 25**	67 vs 75	17 vs 19	8 vs 17	8 vs 8

pre-treatment levels (Figure 3B, 3D). At 2 years from diagnosis, 64% of the patients in both treatment arms, had “not at all” or “a little” complaints of speech and the percentage of patients with severe hoarseness, severe troubles talking to other people or troubles talking on the telephone (all score 4) was 14%. Seventy-nine percent of patients had “not at all” or “a little” swallowing problems. The use of a feeding tube at 2 years from diagnosis was limited to 5% of all patients. There was a trend towards more frequent use of tube feeding in patients age >70 years (3% vs 14%; $P = .07$). None of these items differed significantly between the AR and ARCON treated patients.

Tumor- and patient related factors (T-stage, N-stage, subsite, T-volume, performance status, age and gender) with potential impact on quality of speech and swallowing were analyzed separately (Table 3). At baseline, a clinically significant difference in speech was observed in favor of T2-3 versus T4-stage ($P < .01$) and supraglottic versus glottic tumors ($P < .01$). Quality of swallowing at baseline was negatively influenced by the presence of pathological lymph nodes ($P < .01$), supraglottic tumor site ($P < .01$), larger primary tumor volume ($P < .01$), female gender ($P < .01$), and impaired performance status ($P < .05$). Female patients had more speech and swallowing problems at completion of radiotherapy, especially when receiving ARCON ($P < .01$). During the two years after treatment swallowing problems settled and decreased to baseline levels, except in the elderly (> 70 years of age) group (at 24 months, $P < .01$). In patients presenting with T4 tumors long-term function of speech and swallowing was not impaired compared to those with T2-T3 tumors (speech: 65% vs 64%; $P = 1.0$ and swallowing: 75% vs 80%; $P = 0.57$).

Discussion

This randomized trial, comparing accelerated radiotherapy alone with accelerated radiotherapy in combination with carbogen and nicotinamide for locally advanced laryngeal cancers, revealed similar changes in HRQoL for both treatment arms. After a transient period of clinically significant impairment for the majority of items in the core measure Questionnaire-C30 (QLQ-C30, version 3) and the disease-specific Head & Neck cancer module (QLQ-H&N35), a return to baseline levels was observed after 6 months for the majority of HRQoL items. However, subjective improvement of symptoms over time was less pronounced for dry mouth, and associated symptoms of sticky saliva and altered taste and smell. This pattern of HRQoL scores after treatment for head and neck cancers of various subsites has been observed by others as well (1,6,16).

The main goal of primary radiotherapy for laryngeal cancer is to preserve speech and swallowing functions. However, preservation of organ anatomy does not necessary translate into preservation of organ function. Comprehensive and long-term data on organ function from prospective trials are sparse (3,4,26). Improving with time, at 2 years after diagnosis the majority of patients treated in the current trial had no or just few complaints of speech whereas the percentage of patients with severe problems was less than 15%. Significantly impaired speech or voice quality at 2 years was reported in 3% to 8% of patients in the RTOG 91-11 trial, without significant difference between the three regimens (3,4). These percentages compare favorably to the 14% in the current study. The difference in favour of the RTOG 91-11 trial might be explained by the use of a different questionnaire and/or a higher rate of glottic tumors in the ARCON trial. Around 80% of patients treated in the current study reported no or just a little complaints of swallowing and only 5% used a feeding tube at 2 years from diagnosis. Feeding tube data were not collected in the RTOG 91-11 study, but inability to swallow was reported in less than 3% of patients (3,4). At 1 and 5 years from diagnosis, the RTOG-study 90-03 reported a much higher rate of tube feeding dependence of 13% after accelerated radiotherapy in a cohort of patients with head and neck cancer of various subsites (27). Conventional 3D-radiotherapy techniques were used for patients treated in the ARCON and RTOG-trials. With state-of-the-art intensity-modulated radiotherapy (IMRT) techniques, reduction of the radiation dose to uninvolved swallowing structures can be obtained. There is increasing evidence that IMRT results in a reduction of late swallowing toxicity and an improvement of patient functional outcomes (28,29). Thus, with current application of modern high-precision radiotherapy techniques it can be expected that functional outcome will further improve while tumor control rates remain high.

While organ preservation positively affects HRQoL, xerostomia does not (30). Radiation induced xerostomia is the most commonly reported late side-effect of radiotherapy to the head and neck region (31). At 6 months, a return to baseline levels was observed in our study for the majority of HRQoL items with exception of dry mouth, and associated symptoms of sticky saliva and altered taste and smell. Similar outcomes were found by Nutting et al. who randomized patients with mainly oropharyngeal cancers between conventional radiotherapy and parotid sparing IMRT (2). At 2 years, grade ≥2 xerostomia was observed in 83% vs 29% of patients receiving conventional radiotherapy vs. IMRT, respectively. It is expected that the 30% rate of moderate to severe xerostomia, observed in the current study, will decrease with the use of IMRT.

Potential patient- and tumor-related prognostic factors for functional outcome after larynx preservation were also studied. At baseline, more advanced T-stage, subsite,

presence of pathological lymph nodes, impaired performance status and female gender were correlated with impaired speech and/or swallowing. In all patients there was a complete remission of the primary tumor at the time of analysis resulting in significant improvements of these functions with time after treatment and at two years none of these clinical factors were correlated with larynx function anymore. Age older than 70 was the only factor associated with impaired recovery of swallowing function. Similar observations in older age groups have been made by others (9,32). Nevertheless, it should be mentioned that the vast majority of patients older than 70 had a good functional outcome.

In patients presenting with T4 tumors, many head and neck oncologists are reluctant to use (chemo)radiotherapy and still prefer laryngectomy. Although chemotherapy can add to the toxicity profile of radiotherapy, this concern is mainly based on lower expected disease control rates and anticipated poor functional outcome (9,33,34). Recently, Rosenthal et al. demonstrated that the initial loco-regional control rate in a large cohort of 221 patients with T4 laryngeal cancer, was superior with upfront laryngectomy compared to larynx preservation approaches with chemoradiotherapy (34). However, ultimate loco-regional control was comparable because of the high percentage of successful surgical salvage procedures. Based on our data on long-term speech and swallowing, outcome of patients presenting with T4 tumors was not impaired compared to those with T2-3 tumors. A function preserving approach should be first choice for every patient with a larynx carcinoma, patients with selected T4 tumors included. However, surgery is probably a better alternative for cases with extensive cartilage destruction and tumor spread into the soft tissues of the neck where poor functional outcome is expected. Also patients who present with severe stridor while adequate endoscopic debulking is not possible are probably better served with laryngectomy (35). Merely the classification “T4” does not implicate that larynx preservation should be dismissed as treatment option. The current trial also demonstrated that local control rate for patients with T4 tumors was 73% at 5 years, which was not significantly different from T2 and T3 tumors (17). Recently, several reports confirmed that laryngeal preservation, loco-regional control, and overall survival in patients with selected T4 tumors are similar to patients with less advanced primary tumors (36,37).

Conclusion

Quality-of-life reports of patients with T2-4 larynx carcinoma were good after treatment with accelerated radiotherapy with or without carbogen and nicotinamide. AR and ARCON resulted in high local tumor control rates (± 80%) and a benefit of 8% in

regional control rate with ARCON. After both regimens excellent speech and swallowing function could be maintained for the majority of patients, selected T4-stages included. Long-term dry mouth, sticky saliva and changes in taste and smell were limited to one quarter of patients. Older age was associated with impaired recovery of swallowing function.

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7

General Discussion and future perspectives

Geert O. Janssens

General Discussion

Functional larynx preservation is a main goal when treating patients with advanced laryngeal cancer. The purpose of this thesis was to investigate the addition of carbogen breathing and nicotinamide to accelerated radiotherapy (ARCON) in 345 patients with advanced laryngeal cancer.

Positive trial or negative trial?

The outcome of the randomized trial comparing accelerated radiotherapy alone with ARCON is described in Chapter 2. There was no significant difference in primary endpoint. In the experimental arm local control after 64 Gy was consistent with the 80% local control rate obtained in the preceding phase II ARCON trial [1]. Unexpectedly, the local control rate with 68 Gy in the control arm (78%) was higher than the rates reported in retrospective studies at the time of onset of this study. This 4 Gy absolute dose difference to the larynx, to reduce the risk of cartilage damage, is a likely explanation for the lack of additional benefit in local control with ARCON compared to AR. In fact, it supports the enhancing effect of ARCON for local tumor control as similar local control is obtained with a lower radiotherapy dose. In both treatment arms pathologic lymph nodes did receive a same dose of 68 Gy. A benefit of 8% in regional control rate was observed in favor of ARCON. This finding is consistent with the results of a recent systematic review and meta-analysis of hypoxia modification in head and neck cancer in which an absolute risk reduction of 8% for loco-regional control was observed [2].

Five-year larynx-preservation rates of AR and ARCON were 84% and 87%, respectively and equivalent to the 84% observed in the RTOG 91-11 trial with a combination of conventional radiotherapy and concurrent cisplatin 100 mg/m^2 on days 1,22 and 43 [3]. Unfortunately, no adaptation in study design was performed when the outcome of the RTOG 91-11 trial was published in 2003 [4]. As no randomized comparison between AR, ARCON and concurrent radiotherapy with cisplatin for laryngeal cancer is available today, the combination of conventional radiotherapy with concomitant cisplatin remains the standard for patients with advanced laryngeal cancer.

Toxicity

Although pre-clinical and phase I and II trials do suggest an enhancement of acute reactions with ARCON, a similar acute toxicity profile for skin and mucosa-associated symptoms is observed for both regimens. A likely explanation for this observation is the lower boost dose to the larynx in patients treated with ARCON (64 Gy vs 68 Gy) to decrease the risk of cartilage damage. Strikingly, the median duration of confluent mucositis for the ARCON regimen in the multi-center phase III trial was limited to a

median of 3 weeks which is only half of what was observed in previous trials [5,6]. Given the large number of patients enrolled from Nijmegen (N=144/345), a subgroup analysis was performed. In this subgroup, a median duration of confluent mucositis of 5.0 weeks (versus 2.0 weeks in other centers, $P = <.01$) was observed, suggesting that significant inter-observer and inter-institutional variations in toxicity scoring do exist. It is important to be aware of this phenomenon when interpreting data from multi-center studies and this problem is probably of even greater relevance for late toxicity [7].

Full compliance with carbogen breathing and nicotinamide intake was high. Despite, a number of patients treated with ARCON experienced discomfort with the breathing procedure and digestive troubles due to nicotinamide intake, difficult to manage with adjustment of anti-emetics. This resulted in a discontinuation of carbogen breathing and nicotinamide intake for 6% and 12% of patients, respectively. It should be noted that the current study does not give evidence of the benefit for the use of ARCON compared to accelerated radiotherapy and carbogen breathing alone. Whether outcome is improved by the use of nicotinamide will need a three-armed study to decide (radiotherapy alone, radiotherapy with carbogen and radiotherapy with carbogen and nicotinamide). Analysis of late radiation morbidity did not reveal significant differences between AR and ARCON. All patients who developed a cartilage necrosis could be managed conservatively. Four patients received a tracheostomy (AR, n=1; ARCON: n=3), and there was no need for laryngectomy. This incidence corresponds to the 1-2% of cartilage damage seen in literature. Swallowing limited to soft foods and liquid is less frequently observed with AR or ARCON compared to radiotherapy with concomitant cisplatin [4]. Although direct comparison is difficult because of potential bias, a possible explanation can be the higher number of supraglottic tumors and the planned neck dissection in case of cN2a or cN2b tumors. Neck dissection after chemoradiotherapy has been demonstrated to increase the risk of long-term dysphagia and laryngeal dysfunction [8].

Health related Quality-of-life

Treatment intensification has the potential to increase toxicity [8]. A balanced decision-making between increased toxicity and modest improvements in tumor control is often difficult for patients and treating physicians. So, proper knowledge of Health related quality-of-life (HRQoL) is essential to understand the real benefit of any new regimen. For this reason The European Organisation for Research and Treatment of Cancer (EORTC) Questionnaire-C30 (QLQ-C30) and the Head&Neck cancer module (QLQ-H&N35) was integrated in the ARCON phase III study. Similar changes in HRQoL were observed for both treatment arms: after a transient period of clinically significant impairment for the majority of items, a return to baseline levels was

observed after 6 months for nearly all items with exception of dry mouth, and associated symptoms of sticky saliva and altered taste and smell.

The primary goal of radiotherapy for advanced laryngeal cancer is to preserve speech and swallowing. At 2 years from diagnosis, the majority of patients treated in the current trial had no or just few complaints of speech whereas the percentage of patients with severe problems was less than 15%. A lower percentage of patients with severe problems of speech is observed for patients treated with radiotherapy and concomitant cisplatin within the RTOG 91-11 trial [3,4]. However, this difference might be explained by the use of a different questionnaire and/or a higher rate of glottic tumors in the ARCON trial. Further, 80% of patients treated in the ARCON phase III study reported no or just a little complaints of swallowing and only 5% used a feeding tube at 2 years from diagnosis. The RTOG-study 90-03 compared different radiotherapy fractionation regimens and reported a 13% rate of tube feeding dependence after accelerated radiotherapy in a cohort of patients with head and neck cancer of various subsites [9]. In both RTOG and ARCON trials conventional 3D radiotherapy techniques were used. There is increasing evidence that modern high-precision radiotherapy techniques will result in a further improvement of functional outcome, by reducing the radiation dose to uninvolved swallowing structures [10,11].

Patient selection for hypoxia-modifying therapy

Evidence suggests that hypoxic tumors benefit most from hypoxia-modifying therapy [12-14]. In a translational side-study of the ARCON trial the value of pimonidazole, an exogenous marker for tumor hypoxia, was assessed. Regional control in the group with a high hypoxic fraction was significantly improved with ARCON compared to AR (100% vs. 55%; $P = .01$), whereas no difference between the treatment arms was observed in the group with a low hypoxic fraction. A trend towards a better overall survival was observed for patients with hypoxic tumors in the ARCON arm ($P = .08$). This predictive value of pimonidazol is in line with previous observations by Kaanders et al. [12].

Several other strategies of patient selection for hypoxia-modifying treatments have been attempted over the last years. The DAHANCA-5 trial found that the hypoxia radiosensitiser nimorazole significantly improved outcome of radiotherapy for patients with all subtypes of head and neck cancer compared to placebo [13]. In a translational side-study the value of osteopontin, an endogenous marker for tumor hypoxia, was assessed. Loco-regional control and disease specific survival in patients with high osteopontin plasma levels was significantly improved with nimorazole compared to placebo. Unfortunately, no correlation between high levels of osteopontin and inferior outcome was observed in the TROG 02-02 phase III trial, randomizing patients with

stage III/IV head and neck cancer to radiotherapy and concomitant cisplatin or cisplatin plus tirapazamine, a hypoxic cell cytotoxin [15]. The same trial also failed to demonstrate a benefit of hypoxia modification in an unselected group of patients [16]. However, an interesting substudy provided evidence of a correlation between hypoxia on FMISO-PET-imaging and loco-regional failure [14]. Loco-regional failure in patients with hypoxic tumors receiving cisplatin/fluorouracil was significantly higher compared to patients receiving cisplatin plus tirapazamine.

Approaches using multiplex markers such as gene signatures potentially better reflect the complex cellular response to hypoxia and account for the intra-tumor heterogeneity of hypoxia [17,18]. Based on a 15-gene hypoxia classifier, only HPV-negative patients with hypoxic tumors participating in the DAHANCA-5 trial profited from the addition of nimorazole. This resulted in a significantly better loco-regional control and disease-specific survival [17,19]. More recently, Eustace et al. used a 26-gene hypoxia signature in a cohort of 157 patients with T2-T4 laryngeal cancers treated in the phase III ARCON trial [18]. High expression (>median) was associated with a significant improvement in regional control when treated with radiotherapy plus hypoxia modification versus radiotherapy alone. Evaluation of these findings within a prospective trial is needed.

Tumor volume

Several retrospective studies demonstrate that the macroscopic primary tumor volume, as calculated from pre-treatment CT-scan or MRI, correlates with local control in patients with head and neck cancer treated with definitive (chemo) radiotherapy [20-23]. The University of Florida repeatedly demonstrated that tumors with pre-treatment CT-based volumes smaller than 3.5 cm^3 for glottic and 6.0 cm^3 for supraglottic tumors had a significantly better local control rate [24,25]. However, this strong prognostic factor of primary tumor volume was not confirmed in patients treated with accelerated radiotherapy with or without carbogen breathing and nicotinamide. This study also failed to demonstrate a correlation between local tumor control and T-stage. One possible explanation for the absence of a volume-effect in the current study might be the reduction of overall treatment time by the use of accelerated radiotherapy. Larger tumors might be more hypoxic in absolute volume and more cancer stem cells might start repopulation after radiation induced re-oxygenation. Another important reason can be explained by the methodology: a large cohort of tumors was treated in a homogenous way by 3D-conformal radiotherapy in thermoplastic masks and position verification. Although tumor volume calculations in the retrospective studies are based on diagnostic CT- or MR-imaging, the radiotherapy planning was performed on conventional 2D equipment. It might be that larger tumors in particular are subjective to local failures by underestimating

tumor extension due to 2D-treatment planning and lack of position verification systems.

Less data are available with respect to the volume of involved lymph nodes and nodal control in head and neck cancer. Although the presence of pathological lymph nodes in the current study was a poor prognostic factor for regional control, no correlation between *total nodal volume* and regional control was observed in both treatment arms. This could be explained by the high regional control rates and the limited number of events. To increase the study size and number of events, an additional analysis per individual lymph node was performed. This time, an inferior 5-year nodal control rate was observed in both treatment arms for increasing nodal volumes. The additional analysis also demonstrated that ARCON improved nodal control compared to accelerated radiotherapy alone independently of nodal volume. This observation supports the idea of hypoxia as a cause of regional failure. In contrast, no benefit of regional control was observed in the DAHANCA 6&7 trial by increasing the number of fractions per week from 5 to 6, suggesting only a minor contribution of repopulation in the neck nodes [26].

T4 tumors?

In patients with T4 tumors, anticipated poor functional outcome and lower expected disease control are common reasons to prefer laryngectomy instead of (chemo) radiotherapy. However, based on our data on local control, long-term speech and swallowing, outcome of patients presenting with T4 tumors was not impaired compared to T2-3 tumors. Of course, there is a selection bias in the T4-category as patients with severe stridor, without adequate endoscopic debulking options, were excluded. Expert opinion says that surgery is probably a better alternative for cases with extensive cartilage destruction and tumor spread into the soft tissues of the neck where poor functional outcome is expected [27]. Nevertheless, “T4” does not implicate that larynx preservation should be dismissed as treatment option.

Anemia

Low pre-treatment hemoglobin levels are a strong prognostic indicator of poor disease control and survival in patients presenting with head and neck cancer [28]. Randomized trials failed to demonstrate outcome improvement with transfusions, and erythropoietin was even counterproductive [29-31]. The same observations are done in the current study for patients treated with accelerated radiotherapy alone. However, the impaired outcome of patients with anemia is no longer observed when carbogen and nicotinamide are added to accelerated radiotherapy. This observation is in line with an earlier proposed hypothesis from the ARCON phase II trial [32].

The success of ARCON for patients with anemia can be explained by the so-called "reduced cord radius" [33]. The thickness of the tumor cord radius, being a blood vessel and its dependent tumor cell volume, decreases under anemic conditions [34]. Correction of hemoglobin levels by blood transfusion or erythropoietin will improve oxygenation of tumor cells in the peripheral zone of the cords. However, tumor cords will adapt and begin to proliferate and will once again outgrow their oxygen supply. As a consequence, the benefit of a blood transfusion is transient. In contrast, adaptive mechanisms do not occur with ARCON because the oxygenation increase is too short and only for the duration of the radiation treatment. Shorter oxygen diffusion distances and higher levels of free oxygen in plasma explain how ARCON can exploit adaptive mechanisms in patients with anemia.

There are a number of hypotheses why patients with anemia have an inferior outcome compared with patients without anemia. It is assumed that impaired tumor oxygenation results in a more aggressive and treatment resistant tumor phenotype [35]. Correlations were found between hemoglobin level and tumor hypoxia, measured by pO_2 electrodes, particularly in patients with severe anemia [35-37]. In the current study, however, no correlation was found between hemoglobin and tumor hypoxia, as measured by pimonidazole binding. Albeit that there was only one patient with severe anemia in our study, this observation is consistent with the assertion that distribution, as function of the distance to the vessels, and not the absolute amount of hypoxia is relevant for the response to ARCON. Another reason for the poor prognosis of patients with anemia can be anaerobic glycolysis. Anemic patients have decreased blood viscosity and increased plasma flow. The greater availability of glucose will fuel the process of malignant progression as many tumors rely on glycolysis. This can explain the observation of an association between anemia and the trend toward a higher N-stage in this study. Apart from a causative factor, anemia might also be an epiphenomenon of aggressive tumor behavior. Cytokines inhibit erythropoiesis, affect the life span of erythrocytes and impair iron metabolism [37]

Future perspectives

The addition of carbogen breathing and nicotinamide to accelerated radiotherapy produced a significant gain in outcome for a subgroup of patients with advanced laryngeal cancer. Furthermore, ARCON produced equal levels of toxicity and Quality-of-life compared to accelerated radiotherapy alone. Patients presenting with (larger) pathological lymph nodes and a high hypoxic fraction on pimonidazol staining or patients with pre-treatment anemia profit most of ARCON. Before implementation in daily practice, these findings need to be evaluated in a randomized clinical trial.

However, a number of reasons (e.g. the development of hypoxia gene signatures, lack of a universal measure of clinical hypoxia, new targeted therapies, best standard treatment arm, financial and political issues,…) have thus far hampered the development of a prospective trial.

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8

Summary

Geert O. Janssens

Summary

Hypoxia (the lack of oxygen) and tumor cell repopulation are two important mechanisms of radiotherapy resistance in head and neck cancer. In this thesis the addition of hypoxia modification to accelerated radiotherapy was investigated as a treatment for advanced laryngeal cancer. From 2001 till 2008, 345 patients with cT2-4 laryngeal cancer were randomly assigned to accelerated radiotherapy (AR) or accelerated radiotherapy combined with carbogen breathing and nicotinamide (ARCON). Outcome, toxicity profile, role of anemia, impact of tumor volume and quality-of-life were evaluated.

Chapter 1 is the general introduction to this thesis. Since the early nineties, functional larynx preservation is the preferred treatment for patients with advanced laryngeal cancer. However, a significant subset of patients will develop a loco-regional disease recurrence. Tumor cell repopulation and hypoxia are considered important mechanisms of therapy failure. Although during a course of radiotherapy the number of tumor cells with clonogenic potential is greatly reduced, cells that survive are triggered to repopulate more effectively. In addition, impaired blood perfusion and increased diffusion distances due to a chaotic vascular system within tumors will result into large temporal and spatial variability in tumor oxygenation, and consequent hypoxic radioresistance. ARCON has been developed to counteract repopulation by delivering several radiotherapy fractions per day, and hypoxia by inhalation of a hyperoxic gas and administration of nicotinamide to decrease diffusion- and perfusion-limited hypoxia, respectively. This chapter focuses on the effect of carbogen breathing and nicotinamide observed in preclinical studies using mouse mammary tumors.

Phase I studies demonstrated the feasibility of ARCON. In a large phase II trial local control rates of 80% were observed for advanced-laryngeal cancers. The high local control rate for advanced laryngeal cancers, approaching the results reported for T1 and T2 lesions, supported the concept of increased susceptibility of tumors to the biologically based approach of ARCON. However, large heterogeneity in biological characteristics does exist between tumors of the same site and histology. For this reason, markers of proliferative activity (Ki-67) and oxygenation status (e.g. pimonidazole, CAIX) in tumors become important and are discussed as a potential approach to select candidates for ARCON.

Chapter 2 reports on the outcome of the randomized trial. Patients with T2 glottic carcinoma with impaired cord mobility or subglottic extension, T2 supraglottic carcinoma with invasion of the mucosa of the base of tongue or vallecula or invasion of the medial wall of the piriform sinus, T3-4 glottic or supraglottic carcinoma at any

N-stage were eligible. A dose of 68 Gy in 34 fractions of 2 Gy over 5.5 weeks was prescribed to the primary tumor and macroscopic involved lymph nodes. To limit the risk of laryngeal necrosis, ARCON patients received 64 Gy on the laryngeal cartilage. The primary endpoint of the study was local control. After a median follow-up of 44 months, local tumor control rate at 5 years was 78% for AR vs. 79% for ARCON (P= .80), with larynx preservation rates of 84% and 87%, respectively (P= .48). The 5-year regional control was significantly better with ARCON compared to AR (93% vs. 86%, P=.04). In a translational side study, the hypoxia marker pimonidazole was used to assess the oxygenation status in tumor biopsies. The improvement in regional control was specifically observed in patients with hypoxic tumors and not in patients with well-oxygenated tumors (100% versus 55% respectively, P= .01). *Thus, despite lack of benefit in local control, a significant gain in regional control was observed with ARCON. Translational research using a hypoxia marker assay demonstrated that proper patient selection is key to the success of this approach.*

Acute toxicity was scored weekly from baseline until week 8 plus every 2-4 weeks thereafter and described in **Chapter 3**. Between both treatment arms (AR vs. ARCON) no statistically significant difference was observed for incidence of acute skin reactions (moist desquamation: 56% vs. 58%, P= .80), acute mucosal reactions (confluent mucositis: 79 vs. 85%, P= .14) and symptoms related to mucositis (severe pain on swallowing: 53% vs. 58%, P= .37; nasogastric tube feeding: 28% vs. 28%, P= .98, narcotic medicines required: 58% vs. 58%, P= .97). Although statistically significant, the median duration of confluent mucositis was only slightly different in favor of AR (2.0 vs 3.0 weeks, P= .01). There was full compliance with carbogen breathing and nicotinamide in 86% and 80% of the patients with discontinuation in 6% and 12%, respectively. Adjustment of anti-emesis prophylaxis was needed in 42% of patients. *These observations provided a convenient base for determination of the real therapeutic benefit of ARCON.*

In **Chapter 4**, the outcome of a subgroup of 54 patients, participating in the phase III trial, who presented with anemia is discussed. From literature, it is well known that anemia is associated with poor tumor control and prognosis. Previously, it was demonstrated in retrospective analyses that ARCON can correct this adverse outcome in patients with head and neck cancer. Pretreatment hemoglobin levels were available and below normal in 27/173 and 27/167 patients treated with AR and ARCON, respectively. In patients with normal pre-treatment Hb-levels, treatment with ARCON had no significant effect on 5-year loco-regional control (79% vs. 75%, P= .44) and disease-free survival (75% vs. 70%, P= .46) compared to AR. However, in patients with low pre-treatment Hb-levels ARCON significantly improved 5-year loco-regional control (79% vs. 53%, P= .03) and disease-free survival (68% vs. 45%,

P= .04). In multivariate analysis including other prognostic factors, pre-treatment Hb remained prognostic for loco-regional control and disease-free survival in the AR treatment arm. No correlation was observed between pre-treatment Hb-levels and pimonidazole uptake in tumor biopsies. *The observation of improved outcome in patients with anemia supported previous findings from the phase II ARCON trial. However, the potential of ARCON to correct the poor outcome of patients with anemia should be further explored and validated in a randomized trial with focus on patients presenting with anemia.*

Retrospective studies indicate that larger tumor volume is a strong prognostic indicator for poor tumor control after (chemo)radiotherapy for laryngeal cancer. The impact of tumor volume on the outcome of patients with locally-advanced laryngeal cancer treated in the phase III ARCON trial was investigated in **Chapter 5**. Pre-treatment CT-scans of 270 patients were available for tumor volume calculation. Contouring of the primary tumor and involved lymph nodes was reviewed. Of 137 and 133 treated with AR or ARCON, 57 and 80 vs. 56 and 77 patients had glottic and supraglottic tumors, respectively. A correlation between primary tumor volume and T-stage was observed (Rs= .51, *P*= <.01). In neither of the treatment arms a correlation was detected between the primary tumor volume and local control, regional control or metastasis-free survival. A strong correlation between total nodal volume and N-stage was found (Rs= .93, *P*= <.01). Both in the AR and ARCON groups total nodal volume was not associated with poorer regional control rate. However, based on individual lymph node analysis, nodal control was in favor of ARCON, irrespective of volume (*P*= <.01). *The strong prognostic value of primary tumor volume, observed in a number of retrospective analyses was not confirmed in patients treated in this prospective randomized trial with 3D-conformal, accelerated radiotherapy ± carbogen breathing and nicotinamide. In patients with pathological lymph nodes, a correlation between total nodal volume and regional control was lacking as well. Additional analyses based on individual nodal volumes demonstrate an excellent regional control rate and a significant benefit of ARCON.*

Radiotherapy for patients with advanced laryngeal cancer has a significant impact on health-related quality-of-life (HRQoL), as reported in **Chapter 6**. The price of treatment intensification is a potential increase of acute and long-term toxicity. Between AR and ARCON, no difference in toxicity has been observed. However, proper knowledge of HRQoL is equally essential to understand the real benefit of this new regimen. For this reason, HRQoL was assessed using the European Organisation for Research and Treatment of Cancer (EORTC) HRQoL Questionnaire-C30 (QLQ-C30) and the Head & Neck cancer module (QLQ-H&N35) at baseline, at completion of radiotherapy and at 6, 12, and 24 months post-baseline. Moderate to severe clinical impact of the

treatment was observed for nearly all items of the QLQ-C30 and QLQ-H&N35 between baseline and end of treatment. Again, no difference in HRQoL score between AR and ARCON was observed. At 6 months, scores returned to baseline level with exception of dry mouth, sticky saliva, and taste/smell. At 2 years from baseline, the percentage of patients reporting moderate to severe complaints of dry mouth, sticky saliva, or changes in taste/smell was 30%, 22% and 18%, respectively while the majority of patients had no or few complaints of swallowing (79%) or speech (64%). The use of a feeding tube at 2 years from diagnosis was limited to 5% of patients. *It was concluded that HRQoL reports were similar with AR or ARCON. After both regimens excellent speech and swallowing function could be maintained for the majority of patients, selected T4-stages included. Long-term dry mouth, sticky saliva and changes in taste and smell were limited to one quarter of patients.*

9

Samenvatting

Samenvatting

Hypoxie (zuurstofgebrek) en tumor cel repopulatie zijn twee belangrijke mechanismen die de kans op recidief na radiotherapie voor een tumor van het hoofdhals gebied kunnen vergroten. In dit proefschrift werd de rol van hypoxie correctie onderzocht bij de behandeling van het gevorderd larynx carcinoom. Tussen 2001 en 2008 werden 345 patiënten met een cT2-T4 larynx carcinoom gerandomiseerd tussen enerzijds geaccelereerde radiotherapie (AR), anderzijds geaccelereerde radiotherapie in combinatie met inhaleren van carbogen gas (98% O_2 + 2% CO_2) en slikken van capsules met nicotinamide (ARCON). De uitkomst, het toxiciteitsprofiel, de rol van anemie, de bijdrage van het tumorvolume en de levenskwaliteit werden onderzocht.

Hoofdstuk 1 omvat de algemene inleiding tot dit proefschrift. Sinds enkele decennia, is het behoud van spraak- en slikfunctie een primair streven bij patiënten met een gevorderd larynxcarcinoom. Desalniettemin zal een aanzienlijk aantal patiënten tijdens de eerste jaren na bestraling toch ziekterecidief ontwikkelen. Repopulatie van de tumor cellen en hypoxie worden beschouwd als belangrijke onderliggende mechanismen voor het falen van deze behandeling. Hoewel het aantal tumor cellen dat in staat is om te delen tijdens de bestraling afneemt, is bekend dat overlevende tumor cellen geprikkeld worden om sneller te gaan delen. Daarnaast is een tumor opgebouwd uit een chaotisch kluwen van bloedvaten. Verminderde perfusie en toegenomen diffusie afstanden zorgen voor een belangrijke variatie qua tumor oxygenatie in tijd en ruimte, met hypoxie gerelateerde resistentie als gevolg. ARCON werd destijds ontwikkeld om beide mechanismen te neutraliseren: *repopulatie* door middel van het geven van meerdere bestralingsfracties per dag en *hypoxie* door inhaleren van carbogen en slikken van tabletten met nicotinamide om respectievelijk diffusie- en perfusie-gerelateerde hypoxie op te heffen.

Dit hoofdstuk behandelt kort het effect van carbogen en nicotinamide in preklinische studies. Aansluitend volgen de klinische data. Fase-1 studies toonden de haalbaarheid van ARCON. In een grote fase-2 studie werd met ARCON een lokale controle van 80% bekomen bij patiënten met gevorderde larynxcarcinomen. Een controle percentage bij gevorderde larynxtumoren dat het resultaat van kleine larynxcarcinomen (cT1-T2) benaderde, maakte het larynxcarcinoom een geschikte tumorsite voor biologisch gestuurd onderzoek middels een fase-3 studie. Wat de biologische kenmerken betreft, is het welbekend dat er tussen tumoren van eenzelfde tumorsite en histologie een grote spreiding kan voorkomen. Markers gerelateerd aan tumor proliferatie (Ki-67) en oxygenatie (pimonidazole, CAIX) worden besproken als mogelijke factoren om de geschikte kandidaat voor een therapie met ARCON te selecteren.

Hoofdstuk 2 rapporteert de uitkomst van de fase-3 studie. Patiënten met een T2 tumor van de ware stemband met verminderde beweeglijkheid van een larynxhelft of uitbreiding in de subglottis, T2 supraglottis tumoren met invasie van het slijmvlies van de tongbasis of vallecula of mediale zijde van de sinus piriformis, alsook T3-4 glottis of supraglottis carcinomen, onafhankelijk van de N-status, waren geschikt voor randomisatie. Op de primaire tumor en de aangedane klieren werd een dosis van 68 Gy in 34 fracties van 2 Gy over een periode van 5.5 weken gegeven. Teneinde het risico op larynx necrose te beperken, werd bij patiënten behandeld met ARCON de dosis op de primaire tumor gereduceerd tot 64 Gy. Lokale controle was het primaire eindpunt van de studie. Met een mediane follow-up van 44 maanden, bedroeg de lokale controle na 5 jaar 78% voor AR en 79% voor ARCON ($P=$ .80) en het larynx preservatie percentage respectievelijk 84% en 87% ($P=$ .48). De regionale controle na 5 jaar toonde een significant verschil ten voordele van ARCON (93% vs. 86%; $P=$ .04). Om de oxygenatie status te bepalen van de primaire tumor werd de hypoxie marker pimonidazole gebruikt. Translationeel onderzoek liet een significant voordeel in regionale controle zien bij patiënten met hypoxische tumoren die behandeld werden met ARCON (ARCON vs. AR: 100% vs. 55%, $P=$ .01). Bij tumoren zonder hypoxie werd geen winst van ARCON aangetoond. *Samengevat, ondanks het ontbreken van winst in lokale controle, werd een significant verschil in regionale controle aangetoond bij patiënten behandeld met ARCON. Het gebruik van een hypoxie marker is essentieel om de ideale kandidaat voor een behandeling met ARCON te selecteren.*

De acute toxiciteit werd wekelijks gescoord vanaf aanvang behandeling tot week 8, aansluitend iedere 2-4 weken, en werd beschreven in **hoofdstuk 3**. Tussen beide behandelarmen (AR vs. ARCON) werd geen significant verschil aangetoond qua huidreacties (nattende desquamatie: 56% vs. 58%, P= .80), slijmvliesreacties (confluente mucositis: 79% vs. 85%, P= .14) of symptomen gerelateerd aan de slijmvliesreacties (ernstige pijn bij slikken: 53% vs. 58%, $P=$ .37; voeding via neusmaagsonde: 28% vs. 28%m $P=$ .98; gebruik opioiden: 58% vs. 58%, $P=$ .97). Hoewel statistisch significant, bedroeg het verschil in mediane duur van de confluente mucositis slecht 1 week (AR vs. ARCON: 2.0 vs. 3.0 weken, $P=$ .01). Volledige compliantie met het inhaleren van carbogen en slikken van nicotinamide tabletten bedroeg respectievelijk 86% en 80% terwijl het stopzetten van deze middelen slechts bij 6% en 12% van de patiënten werd geobserveerd. Aanpassing van anti-emetica profylaxe was noodzakelijk voor 42% van de patiënten. *Kortom, het ontbreken van een significant verschil in acute toxiciteit vormt een goede basis om de echte winst van ARCON te beoordelen.*

Hoofdstuk 4 laat de uitkomst zien van 54 patiënten met anemie bij diagnose, behandeld in de fase-3 ARCON studie. Publicaties hebben herhaaldelijk aangetoond dat anemie geassocieerd is met een slechtere lokale controle en prognose. Een eerder uitgevoerde retrospectieve analyse van patiënten met een tumor in het hoofdhals gebied, liet echter zien dat deze inferieure prognose gecorrigeerd kan worden met ARCON. In de fase-3 studie was het hemoglobine (Hb)-gehalte bij diagnose bekend en te laag bij 27/173 en 27/167 patiënten behandeld met respectievelijk AR en ARCON. Bij patiënten met normale Hb-waarden voor de start van de behandeling, werd qua locoregionale controle (79% vs. 75%, $P= .44$) en ziektevrije overleving (75% vs. 70%, $P= .46$) na 5 jaar geen effect gezien van ARCON, vergeleken met AR. Daarentegen werd bij patiënten met een laag Hb bij diagnose en behandeld met ARCON een significante winst in locoregionale controle (79% vs. 53%, $P= .03$) en ziektevrije overleving (68% vs. 45%, $P= .04$) gezien na 5 jaar. Bij multivariate analyse bleef het Hb-gehalte bij diagnose in de AR-arm zijn prognostische waarde voor locoregionale controle en ziektevrije overleving behouden. Er werd geen correlatie aangetoond tussen het Hb-gehalte bij diagnose en de pimonidazole opname in de biopten. *Het aantonen van winst in locoregionale controle en ziektevrije overleving bij patiënten met anemie bevestigt eerdere bevindingen uit de fase-2 ARCON studie. Deze potentieel corrigerende rol van ARCON dient verder te worden onderzocht en gevalideerd in een gerandomiseerde studie, gericht op patiënten met anemie bij diagnose.*

Uit retrospectieve studies bleek het volume van een larynxtumor een prognostische factor te zijn voor tumor controle na radiotherapie ± chemotherapie. **Hoofdstuk 5** beschrijft de impact van het tumorvolume op de uitkomst van patiënten met een gevorderd larynxcarcinoom en behandeld in de ARCON fase-3 studie. Bij 270 patiënten uit deze studie was een CT-scan bij diagnose beschikbaar om het tumorvolume te berekenen. De intekening van de primaire tumor en de aangedane klieren werd nagekeken. In deze groep waren respectievelijk 137 en 133 patiënten behandeld met AR of ARCON, en hadden 57 en 80 versus 56 en 77 patiënten een glottis of supraglottis tumor. Een correlatie tussen het primaire tumorvolume en het T-stadium werd aangetoond (Rs= .51, $P= <.01$). In geen van beide studiearmen werd een correlatie aangetoond tussen het primaire tumorvolume en de lokale controle, regionale controle of metastasevrije overleving. Eveneens werd een sterke correlatie aangetoond tussen het totale kliervolume en de N-status (Rs= .93, $P= <.01$). Het totale kliervolume was in beide studiearmen evenmin geassocieerd met de regionale controle. Echter, bij analyse van de individuele klieren werd, ongeacht tumorvolume, bij patiënten behandeld met ARCON een significante winst gezien wat betreft controle van pathologische klieren ($P= <.01$). *Kortom, het tumorvolume als prognostische factor, aangetoond in meerdere retrospectieve studies, werd niet*

bevestigd in deze prospectief gerandomiseerde studie met geaccelereerde radiotherapie ± carbogen-nicotinamide en 3D-conformele planning. Bij patiënten met pathologische klieren ontbrak eveneens een correlatie tussen het totale kliervolume en de regionale controle. Aanvullende analyse gericht op individuele kliervolumes liet bij patiënten behandeld met ARCON een uitstekende regionale controle en een significante winst zien vergeleken met AR.

De impact van een bestralingsbehandeling, voor een gevorderd larynxcarcinoom, op de levenskwaliteit van patiënten wordt beschreven in **hoofdstuk 6**. Intensivering van een behandeling zoals ARCON kan echter een toename van acute en late schade betekenen. Vooralsnog werd tussen AR en ARCON geen verschil in toxiciteit geobserveerd. Meer kennis van de levenskwaliteit was echter noodzakelijk om de reële winst van een nieuwe therapie te begrijpen. Om die reden werden bij diagnose, bij het einde van de radiotherapie en 6,12 en 24 maanden na aanvang therapie twee EORTC-vragenlijsten (QLQ-C30 en QLQ-H&N35) ingevuld. Tussen start en einde van de radiotherapie werd een matige tot ernstige impact op nagenoeg alle items van beide vragenlijsten geobserveerd. Tussen AR en ARCON werd geen verschil in levenskwaliteit aangetoond. Zes maanden na aanvang therapie werd, met uitzondering van een droge mond, kleverig speeksel en een vreemde smaak/geur beleving, een normalisatie tot uitgangswaarde gezien. Twee jaar na de start van de behandeling bedroeg het percentage patiënten met matige tot ernstige klachten van droge mond, kleverig speeksel of vreemde smaak/geur beleving respectievelijk 30%, 22% en 18% terwijl de grote groep patiënten geen of nauwelijks klachten ondervond met slikken (79%) of spreken (64%). Het gebruik van een neusmaagsonde, 2 jaar na diagnose, was beperkt tot 5% van de patiënten. *We kunnen concluderen dat er geen verschil in levenskwaliteit werd aangetoond bij patiënten behandeld met AR of ARCON. Het merendeel van de patiënten vermeldde een uitstekende spraak- en slikfunctie, zo ook geselecteerde patiënten met T4-tumoren. Na 2 jaar ondervond slechts een kwart van de patiënten last van droge mond, kleverig speeksel en een vreemde smaak/geurbeleving.*

Publiekssamenvatting

Het behoud van de spraak- en slikfunctie is een essentieel streven bij patiënten met een gevorderde tumor van het strottenhoofd. Met behulp van radiotherapie kan in het merendeel van de gevallen genezing worden bereikt. Toch ontwikkelt een aantal patiënten een tumorrecidief. Zuurstof tekort in de tumor en toename van de delingssnelheid van de kwaadaardige cellen zijn twee onderliggende mechanismen die de kans op tumorrecidief beïnvloeden. Om de impact van zuurstoftekort bij patiënten met een gevorderde tumor van het strottenhoofd in kaart te brengen werd een internationale studie opgezet. Patiënten kregen radiotherapie of radiotherapie met toevoegen van zuurstof (ademen van carbogen en slikken van tabletten met nicotinamide) als behandeling aangeboden. Ziektecontrole, bijwerkingen, invloed van bloedarmoede, bijdrage van tumorvolume en levenskwaliteit bij deze behandelingen werden in dit proefschrift onderzocht. Dit onderzoek liet zien dat de kans op ziekterecidief in de klieren aanzienlijk kleiner wordt door toevoegen van zuurstof terwijl geen extra bijwerkingen of een verschil in levenskwaliteit werden vastgesteld. Door voorafgaand aan de behandeling ook het zuurstofgehalte in de tumor te bepalen, is het mogelijk om deze patiënten te selecteren, die baat hebben bij het toevoegen van zuurstof. Verder liet dit onderzoek ook zien dat de toegenomen kans op tumorrecidief bij patiënten met bloedarmoede volledig gecorrigeerd kan worden door het toevoegen van zuurstof voor iedere behandeling. De kans op ziekterecidief in deze studie was niet beïnvloed door het tumorvolume.

Publication list

Related to the thesis

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