Radiobiological and quality of life study of conventional and accelerated fractionated radiotherapy in patients with head and neck squamous cell carcinoma: Correlation of efficacy with cell cycle analysis parameters.

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Abstract

Background: In this report, we investigated the prognostic value of the cell cycle analysis parameters of patients with unresectable locally advanced head and neck squamous carcinoma treated with two different radiotherapy regimens. The secondary endpoint was the evaluation of quality of life before and after radiotherapy in both schedules.

Methods: Twenty two patients were randomized to receive either conventional (70 Gy/2 Gy/fr) or accelerated (64.4 Gy/2.3Gy/fr) 3-D Conformal RT. A fine-needle aspiration (FNA) of the primary or gross adenopathy combined with flow cytometry was carried out before any treatment. QLQ-H&N35 questionnaire was assessed in all patients, performed at baseline and a week after radiotherapy.

Results: Finally, specimens from only nine patients were eligible for flow cytometry. The spearman rho correlation showed no statistical significance between the expression malignant cells in the different cell cycle phases and overall survival, except a trend in S phase (rho= -0.54, P=0.088). A significant (p < 0.05, Wilcoxon test) better outcome (pre vs post-RT) was observed in the scales of global QoL H&N 35 at 29 out of the 35 scales in both RT schedules. No statistical difference was found in QoL H&N 35 scales for conventional versus accelerated schedule of radiotherapy (P>0.05, Mann Whitney test). No difference in survival was noted between the two groups (P=0.92, log-rank test). Acute and late radiation induced toxicity was also equivalent in both schedules.

Conclusions: This study identified that both radiotherapy arms were equivalent in terms of QoL and toxicity. The number of cells in S phase correlated negatively but not-significantly with overall survival. A statistical significant improvement of quality of life was observed one month after the end of irradiation in both arms. More patients with eligible for analysis specimens are needed for the extraction of safe results.

Key words: cell cycle analysis; radiotherapy; head and neck cancer; quality of life.

Running Title: radiobiology and quality of life in H&N cancer patients
Introduction

The most common histologic type in head and neck (H&N) tumors is squamous cell carcinoma. In the cancers of oral cavity, oropharynx, hypopharynx and larynx, alcohol and tobacco abuse are frequent etiologic factors.\(^1\)

Radiotherapy is one of the most important treatment modalities for H&N cancers patients, either in a definite way or in combination with surgery and/or chemotherapy. Patients with locoregionally advanced H&N carcinoma are usually managed with a multidisciplinary approach that includes surgery, radiotherapy and chemotherapy. Definitive combined radio chemotherapy is used for patients who have unresectable disease, and for those who are medically inoperable.\(^2\)

FNA represents a powerful tool for diagnosing of head and neck squamous cell carcinomas without the financial costs and the morbidity of excisional biopsy. Assuming the hypothesis that DNA is the critical target in RT, the differential radio-sensitivity of the phases of cell cycle has been already analyzed in radiobiological publications.\(^3,4\) However, the potential impact of cell cycle to treatment outcome has not been reported yet in the literature.

Moreover, the EORTC quality of life questionnaire (QLQ -H&N35) is an integrated system for assessing the health-related quality of life (QoL) of patients with H&N carcinomas participating in clinical trials.\(^5\)

The aim of the present study was to assess the quality of life and the potential correlation of cell-cycle analysis with the treatment outcome of two radiotherapy schedules: a conventional and an accelerated one.

Material and Methods

In the present prospective randomized study, the primary endpoint was the correlation of treatment outcome in terms of OS with the cell-cycle phases. The secondary endpoint was the evaluation of differences in terms of QoL between the two irradiation schedules. The
measurements for the QoL were performed with the EORTC QLQ-H&N35 modules for HNC patients in two different time periods: before any treatment and one month after the end of the radiotherapy.

Inclusion criteria consisted of patients:

a) Patients 18 years of age or older.

b) Inoperable disease (the constitutional state of all patients precluded an operation for medical reasons and/or severe comorbidities).

c) Newly diagnosed moderately advanced head and neck carcinoma.

d) Pathologically proven squamous cell tumor.

e) Receiving RT and regular follow-up at the radiation oncology Unit of Attikon University Hospital.

f) Prospectively randomized selected patients.

g) Completion of the self-reported questionnaire.

After the initial RT, follow up was performed at 2-month intervals for 2 years, at 3-month intervals for another 2 years, and at 6-month intervals thereafter. In our series, tumor progression was defined as occurrence of loco-regional recurrence, metastasis, or death by head and neck cancer. The protocol was reviewed and approved by the Local Ethics Committee/Institutional Review Board. Informed consent for the procedure was obtained from all patients. The patients were randomized to receive either conventional fractionated (70 Gy/2 Gy/35 fractions/5 days/week) or accelerated (64.4 Gy/2.3Gy/28 fractions/5 days/week) locoregional radiotherapy. The randomization was performed by a PC with random number generator of even or odd ones, corresponding to the two schedules. All patients had biopsy proven squamous cell carcinoma in the enlarged cervical lymph nodes or primary tumor by FNA or by excisional biopsy. All patients underwent comprehensive workup including complete physical examination, CT and/or MRI of the head and neck panendoscopy with directed biopsies. The 2010 American Joint Committee on
Cancer (AJCC) staging classification (7th edition) was used in conjunction with the NCCN’s treatment recommendations for Head & Neck Cancer.\(^6,7\) Stage IVA was defined as moderately advanced local/regional disease. Patients were evaluated for treatment-related acute toxicity at a minimum of every seven days during radiotherapy and 1 month thereafter, while the late toxicity was assessed 6-9 months post irradiation. The radiation induced toxicity score was assessed according to the EORTC/RTOG criteria.\(^8\) The clinical responses were evaluated four weeks after patients completed radiotherapy according to response evaluation criteria in solid tumors (RECIST).\(^9\) CT helped in (a) delineation of the different target volumes, (b) determination of critical structures at risk, (c) placement of beams and shaping of apertures, (d) calculation of dose distribution, (e) verification of plan, and (f) evaluation of treatment response. CT was useful in uncooperative patients, when MRI was contraindicated by ferromagnetic aneurysm clips or cardiac pacemakers. MRI offered information about the soft-tissue extent of tumors, skull-base involvement, perineural spread, intracranial and selection of specific 3D imaging planes.

**Cytological analysis**

FNA cytology was carried out by an experienced cytopathologist. Some preparations were necessary before this procedure as no use of aspirin or substitutes for one week before; routine blood tests (including INR) were completed before the biopsy; suspension of blood anticoagulant medications. The skin that over lied the mass was prepared with a prepackaged, sterile, alcohol preparation sponge that contained 70% isopropyl alcohol. Patients were placed supine with the neck slightly extended. After lesion localization, the neck was prepared in a sterile environment and draped. A 21-gauge needle attached to a 10-mL syringe with holder was used. Topical anesthesia was not usually used. Minimum of 3 passes were performed. Part of the aspirated material was placed, smeared, and fixed on glass slides for “on-site” adequacy evaluation. The rest of the aspirated material of the first pass was immediately injected, by rinsing the needle, into
a vial containing 20 ml of the ThinPrep® method proprietary fixative and hemolytic Cytolyt® solution (Hologic, Marlborough, MA) for slide preparation and staining with Papanicolaou, for cytology evaluation. The rest of the passes were rinsed into RPMI 1640 sterile solution (GIBCO, Invitrogen) for immediate Flow Cytometry study. After the procedure, mild analgesics are used to control post-operative pain. The obtained tissue was examined with cell cycle analysis using flow cytometry. The cell cycle analysis was implemented with staining of Propidium iodine and a mixture of cytokeratins with FITC. The whole process was performed using the Cyflow Space (PARTEC) system of particle analysis.\textsuperscript{10} In details, the percentage of cells in each phase of the cell cycle was counted by flow cytometry. The specific technique allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through an optical and/or electronic detection apparatus. A beam of laser light of a single wavelength is directed onto a hydrodynamic focusing stream of fluid. A number of detectors surround the spot where the light beam penetrates the liquid flow: one in line with the light beam, some others perpendicularly to it, and one or more fluorescent detectors. Every particle between 0.2 and 150 micrometers floating in the liquid that passes through the light beam scatters the light to some direction and simultaneously the fluorescent chemicals in the particle or on its surface can be stimulated and emit light of different wavelength than the respected one of the source. The outcome is a fluorescent distribution (FSC), as shown in figure\textsuperscript{1a}. The staining used was Propidium iodine. The quantity of the staining bounded corresponded to the amount of DNA. The intensity of the emitted fluorescence correlated well with the amount of staining bound by the DNA. Following the staining, the samples were assessed in the cytometer, and the DNA histogram was derived in terms of an FL2-Area (total cell fluorescence). As shown in figure\textsuperscript{1b}, the first and highest peak represented the G0/G1 cells (RN1-Gate), while the second and smaller peak represented the G2/M cells (RN3-Gate), and the area between the two peaks the S cells (RN2-Gate). The results of the analysis were expressed as the percentage of S phase cells and the
proliferative index (PI) that indicated the percentage of cells in the S and G2/M phases. The absolute number of cells in certain cell-phase was also assessed.

**Radio-chemotherapy**

Each patient underwent a virtual CT-simulation, in supine position, using dedicated devices. A thermoplastic mask was used for immobilization. The patients were scanned with 3 mm slice thickness in simulation CT scan and the CT datasets were transferred to Prosoma System through DICOM network, for contouring of target volumes and normal structures. The following structures were delineated: GTV (Gross Tumor Volume), CTV (Clinical Target Volume), and PTV (Planning Target Volume). The PTV1 consisted of gross disease, the PTV2 consisted of gross lymph-node involvement and PTV3 consisted of uninvolved nodal stations.

We used the linear-quadratic (LQ) modelling to equate the hypofractionation schedule to the normalised total dose (NTD) if delivered in 2 Gy-fractions.\(^{11}\) Thus, NTD represents the dose given in 2 Gy fractions that would give an equivalent biological effect to the new hypofractionated dose:

\[
N = \frac{D_{\text{new}}}{2 + \frac{\alpha}{\beta}} \left(1 - e^{-\frac{d_{\text{new}}}{\beta}}\right)\]

where, \(D_{\text{new}}\) and \(d_{\text{new}}\) are the total dose and dose per fraction, respectively, for a suggested hypofractionation scheme. NTD was calculated and tabulated for late reacting tissues (\(\alpha/\beta = 3\) Gy) as well as for head and neck cancer (\(\alpha/\beta = 10\) Gy).\(^{12,13}\) The total physical dose was 64.4 Gy. Considering that \(\alpha/\beta = 3\) Gy and \(\alpha/\beta = 10\) Gy, NTD was 68.3 Gy and 66 Gy, respectively.

Dose prescription for PTV1, PTV2 and PTV3 was 70Gy, 64Gy, and 46-50 Gy, respectively. For the accelerated scheme, the relevant physical dose prescription for the above mentioned PTVs was 64.4Gy, 55.2Gy and 43.7Gy, respectively. Spinal cord, parotid gland, brain stem, optic nerves, orbits, cervical esophagus were outlined as dose-limiting structures. To
evaluate the dose constraints for normal tissues, the QUANTEC trial was used, corrected also for accelerated hypofractionation.\textsuperscript{14} Treatment plans were performed with the ECLIPSE treatment planning (VARIAN). Partial wedging or dynamic MLC was employed to improve dose homogeneity. Heterogeneity of \(-5\%\) to \(+7\%\) was acceptable, according to ICRU criteria.\textsuperscript{15} Treatment was delivered daily, five days a week. Weekly portal films were obtained in the treatment position with a therapeutic beam to confirm adequate patient positioning. Patients were treated with a VARIAN linear accelerator of 6 MV (600 C) or 15 MV (2100 C) energy. For the treatment technique, histograms of the targets and organs at risk were generated; a number of parameters, including mean, median and maximum dose, were also evaluated.

In terms of chemotherapy, single agent cisplatin 40 mg/m\textsuperscript{2} intravenously weekly was given concurrently with the irradiation.\textsuperscript{7}

\textit{Quality of life measurements}

Under license from the EORTC QoL group, the QLQ-H&N35 module was used for assessing the QoL for head-and-neck cancer patients.\textsuperscript{5} It incorporates seven multiple-item scales that assessed the symptoms of pain, swallowing ability, senses (taste/smell), speech, social eating, social contact, and sexuality. Also included are six single-item scales, that tested the presence of symptomatic problems, associated with teeth, mouth-opening, dry mouth (xerostomia), sticky saliva, coughing, and feeling ill. A high score for a functional or global health related QoL scale represented a relatively high/healthy level of functioning or global quality of life, whereas a high score for a symptom scale represented the presence of a symptom or problem. The QLQ-H&N35 questionnaire was used at baseline and one month post irradiation.

\textit{Follow-up}

Patients were seen in follow-up 1 month post-irradiation and every 6-8 weeks thereafter in the first year and every 2-3 months in the second year. Physical examination including fiberoptic
laryngoscopy, were performed at each visit. For patients with ambiguous findings on physical examination, from computed tomography (CT), or FDG-PET imaging, biopsies were obtained by fine needle aspiration under ultrasound or CT guidance or by panendoscopy. If biopsies were negative, close follow-up with physical examination and repeated image studies were preferential.

Statistical analysis

Correlation of numerical variables was investigated by spearman-rho correlation coefficient. A Wilcoxon test was used to analyze the differences of QLQ-H&N35 parameters before and after RT. For the differences in the QLQ-H&N35 scores and age between the two groups, a Mann-Whiney test was used. The difference in the incidence of EORTC/RTOG radiation induced toxicity was assessed with chi² test. The survival analysis consisted of Kaplan-Meier curves and log-rank test. Values of P < 0.05 were considered to be statistically significant. The whole analysis was performed by using the SPSS version 10 (Chicago, IL).

Results

Between July 2009 and December 2011, 22 patients (Men: 17, Women: 5) with locally advanced head and neck squamous carcinoma were admitted in the Radiation Oncology Unit of University ATTIKON Hospital. The patients’ mean age was 68 years. As shown in table 1, the primary sites of tumor were: Nasopharynx (n: 4), Oral Cavity / Oropharynx (n: 9), Hypopharynx (n: 1), Salivary Gland (n: 4), Orbit (n: 3) and Larynx (n: 1). Twenty two patients were examined with FNA and cell cycle analysis (Figure 2) before any treatment. Of the available 22 patients for this study, finally a total of 9 patients were eligible for the cell cycle analysis, since in thirteen patients the cytological samples contained only a few amounts of tumor cells. As a result, the analysis of cell
cycle parameters wasn’t feasible for the rest of 13 patients. Of the nine patients with eligible for analysis specimens, 5 patients underwent an accelerated fractionated schedule and 4 patients underwent a conventional fractionated schedule. Of a total of 22 patients 13 patients underwent an accelerated fractionated schedule and 9 patients underwent a conventional fractionated schedule.

The most common diagnostic post-radiation changes on the CT scan were a slightly or moderate thickening and reactive edema of the mucosa and tumor regression by more than two thirds. Because of mucositis and edema FNA couldn’t obtain a sufficed number of tumor cells. We based only on the initial cell cycle analysis results.

Eight patients died of distant metastasis, (6 lung and 2 liver metastasis). One patient died from loco-regional recurrence. The spearman rho correlation showed no statistical significance between the cell cycle phase’s expression and overall survival, with the exception of a trend concerning the S phase (figure 3). The rho values of the correlation of OS with the different phases were: rho[G0/G1]=-0.36 (P=0.27), rho[S]=-0.54 (P=0.088), rho[G2/M]=-0.25 (P=0.45). There was no significant difference in OS by log-rank test between the two groups (P=0.92), as shown in figure 4. The OS for the group of accelerated arm was 17.8 months while for conventional schedule was 17.25 months.

We have also assessed the quality of life of all 22 patients with EORTC QLQ-H&N35 module in two different time periods (before any treatment and a week after the end of radiotherapy), as shown in Table 2. A significant (p < 0.05) better outcome (pre vs. post-RT) was observed in the scales of global QoL H&N 35 at 29 out of the 35 scales in both RT schedules except coughing, hoarseness, feeling sick, speaking, pain medication and feeding tube. The total number of patients that participated in accelerated arm was 13 patients and the number of patients in the conventional schedule was equal to 9 patients. Finally, in terms of QLQ-H&N-35, no statistical difference was found between the two schedules (accelerated versus conventional) of radiotherapy treatments analyzed by Mann- Whitney test (Table 2). All patients in both groups presented complete response
according to RECIST criteria (tumor shrinkage >70%). The acute and late radiation induced morbidity in group A and C is shown in table 3, indicating no significant differences between the two irradiation schedules.

**Discussion**

Data indicate that head and neck squamous carcinomas have an accelerated repopulation and grow rapidly. No fractionation schedule has proven to be ideal for all type of tumors. In radiotherapy alone schedules, the delivery of at least 10Gy per week is strongly recommended, while the increasing of the total irradiation time is correlated with higher recurrence rates. Thus, the clinical radiobiological rationale for using accelerated fractionation in HNSCC is the increase of local control. The tumor time factor with the overall time factor would be negligible for late complications provided a reason for shortening the overall treatment time (i.e., increasing the dose delivered per week). Fu et al. in the four-arm Radiation Therapy Oncology Group (RTOG) 9003 phase III trial showed that accelerated fractionation schedule gave a significantly improved local control compared with conventional fractionation for a comparable incidence of late effects. The cleanest test of accelerated fractionation in HNSCC was probably the DAHANCA trial by Overgaard et al. in which the total dose of 66 to 68 Gy and the 2-Gy fraction size was kept identical in the two trial arms, but acceleration was achieved by delivering 6 fractions per week in the experimental arm versus the standard 5 fractions per week in the control arm, which shortened the overall treatment time by 7 days, from 46 to 39 days. There was no statistically significant increase in late toxicity in the 6 fractions per week arm relative to the 5 fractions per week arm. Thus the DAHANCA trial provides direct evidence, without the possible confounding effect of differences in total dose or dose per fraction, for the importance of the overall time factor in HNSCC and that a therapeutic gain is achievable by treatment acceleration. In this trial they observed a 12% improvement in tumor control probability from 64% to 76% (\[P = 0.0001\]), confirming the potential clinical benefit of shortening the
radiotherapy time. Previous study with accelerated hypofractionated radiotherapy has been already reported by Zygogianni et al., showing promising results in advanced stage of H&N cancer. In our study, in accordance with the DAHANCA trial, we didn’t find any difference in either acute or late toxicity between the two irradiation schedules. However, no difference in either overall survival or treatment response was noted, probably due to the small number of patients.

Ionizing radiation produces its biologic effects by imparting energy to tissues, thereby causing DNA damage and loss of cellular reproductive ability. Some cells die relatively rapidly through apoptosis. However, most cells do not manifest evidence of damage until mitosis occurs, and several divisions may ensue before actual cell death (termed mitotic cell death). For this reason, most tumors do not show immediate shrinkage after starting RT and may take weeks or longer to shrink. Some low-grade, slowly proliferating tumors histologically appear to be viable for prolonged periods after irradiation. Irradiation induces both single- and double-strand DNA breaks, with the double-strand breaks generally considered the lethal event. Because the cell cycle is strongly affected by irradiation, and radiosensitivity depends on cell cycle position and cell cycle progression, it is not surprising, however, that some association between apoptosis and radiosensitivity has been observed. According to Hartwell et al. multiple pathways are involved in the maintenance of genetic integrity after exposure to ionizing radiation, most of which are related to the cell cycle. Since the S-phase is potential the most radioresistant, then obviously there should a relation between the cells in that phase and the treatment outcome. In our study, although not significant, we found a trend of negative treatment outcome and absolute numbers of cancerous cells in S-phase. This paper, for the first time, describes a clinical study evaluating the prognostic role of tumor cell phases comparing the conventional versus accelerated radiotherapy schedules. In our study, there was definitely a trend of negative correlation of cycles in S-phase (the most radio-resistant) with the outcome in survival. However, the number of specimens was really too small to extract safe conclusions. A prospective study with more eligible
specimens stands in need for this purpose. From the other point of view, the two radiotherapy schedules (group A and C) seems equivalent in terms of QoL, radiation induced toxicity and survival. The main message from this aspect is that radiobiology really works.

Conclusions

As a final conclusion it appears that both radiotherapy schedules are equal in terms of overall survival, acute toxicity, loco regional control rate and quality of life. The cell cycle markers studied did not provided additional prognostic information on disease recurrence after initial treatment of head and neck inoperable moderately advanced local regional tumors when the cytopathological parameters were taken into account. However, the trend in terms of the negative correlation of cells in S-Phase with the survival outcome should not be underestimated, by means of the small number of specimens analyzed. At the same time, the quality of life questionnaire of the EORTC QLQHN35 generally showed a statistically significant difference and a better outcome after radiotherapy treatment, while there was no significant impact of the schedule (accelerated versus conventional) to the quality of life. The main limitation of the present study was the small number of patients. However the question is still open: has the radio-resistant phase-S a definite negative impact to the radiotherapy outcome?

Acknowledgements

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Conflict of Interest

All authors disclose any financial or other conflicts of interest that might bias the present work.
References


Legends

**Figure 1.** Trial’s Design.

**Figure 2.** Malignant Cell Cycle Analysis with eligible specimen of an oropharyngeal carcinoma capable for cytology analysis. **a:** fluorescent distribution; **b:** peaks indicating the different cell-phases.

**Figure 3.** Linear regression analysis curve between overall survival and cell population in S-phase (rho=-0.54, P=0.088).

**Figure 4.** Kaplan-Meier curves for the two radiotherapy schedules A (accelerated) and C (conventional), showing a P=0.92 (Log rank test).
Table 1. Characteristics of all patients participating in the study (N=22)

<table>
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<th>Accelerated (N=13)</th>
<th>Conventional (N=9)</th>
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<tr>
<td>Age median (range)</td>
<td>61 (46-76)</td>
<td>67 (54-78)</td>
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<td>Sex (male / female)</td>
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<tr>
<td>IVb</td>
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* Mann-Whitney test

** Chi² test
Table 2. Statistical Analysis of QoL H&N35 parameters measured pre versus post-RT, related also to the radiotherapy schedule of conventional (C) versus accelerated (A).

| Topic                  | Overall | Pre-RT | Post-RT | P   | Pre-RT | Post-RT | P   | Pre-RT | Post-RT | P   |
|------------------------|---------|--------|---------|-----|--------|---------|-----|--------|---------|-----|-----|
| (HN35 items)           | N=22    | N=13   | N=9     |     | N=13   | N=9     |     | N=13   | N=9     |     |
| Pain (1-4)             | 2.9±0.4 | 2.9±0.3 | 2.0±0.4 | <0.001 | 1.9±0.4 | 1.9±0.3 | 0.84 |
| Swallowing (5-8)       | 1.2±0.3 | 1.3±0.3 | 2.5±0.6 | <0.001 | 2.6±0.5 | 2.6±0.3 | 0.79 |
| Teeth (9)              | 1.2±0.4 | 1.2±0.4 | 1.8±0.9 | 0.013 | 1.2±0.5 | 1.6±0.7 | 0.69 |
| Mouth opening (10)     | 1.4±0.5 | 1.4±0.5 | 2.3±0.9 | <0.001 | 1.5±0.5 | 2.7±0.7 | 0.36 |
| Dry mouth (11)         | 1.8±0.4 | 1.7±0.5 | 2.0±0.6 | <0.001 | 1.7±0.5 | 3.2±0.7 | 0.69 |
| Saliva (12)            | 1.8±0.4 | 1.8±0.4 | 1.9±0.3 | <0.001 | 3.1±0.7 | 3.6±0.5 | 0.18 |
| Smell (13)             | 1.7±0.7 | 1.7±0.6 | 2.9±0.6 | <0.001 | 1.8±0.8 | 2.7±0.9 | 0.74 |
| Taste (14)             | 1.5±0.5 | 1.6±0.5 | 3.4±0.6 | <0.001 | 1.5±0.5 | 3.1±0.9 | 0.60 |
| Coughing (15)          | 2.2±0.8 | 2.0±0.8 | 2.3±1.1 | 0.73 | 2.1±0.7 | 1.8±0.9 | 0.29 |
| Hoarseness (16)        | 1.8±0.8 | 1.8±0.8 | 2.1±1.1 | 0.28 | 1.8±0.7 | 2.2±0.8 | 0.74 |
| Fell sick (17)         | 2.7±0.9 | 2.8±0.8 | 2.1±1.0 | 0.059 | 2.7±1.0 | 2.1±0.8 | 0.51 |
| Appearance (18)        | 2.7±0.5 | 2.7±0.5 | 2.1±0.5 | 0.002 | 2.8±0.4 | 2.1±0.6 | 0.89 |
| Eating (19-22)         | 1.8±0.4 | 1.8±0.3 | 2.9±0.5 | <0.001 | 1.7±0.4 | 2.9±0.5 | 0.84 |
| Speaking (23-24)       | 2.0±0.6 | 2.0±0.5 | 2.1±0.7 | 0.46 | 2.3±0.9 | 2.5±0.9 | 0.32 |
| Social contacts (25-28)| 1.8±0.4 | 3.1±0.6 | 3.1±0.7 | <0.001 | 3.1±0.6 | 3.1±0.6 | 0.95 |
| Sexuality (29-30)      | 1.8±0.5 | 1.7±0.4 | 2.9±0.9 | <0.001 | 3.1±0.4 | 3.3±0.4 | 0.60 |
| Pain medication (31)   | 1.3±0.5 | 1.4±0.5 | 1.5±0.5 | 0.088 | 1.6±0.6 | 1.9±0.6 | 0.16 |
| Food additives (32)    | 1.1±0.4 | 1.1±0.3 | 1.8±0.4 | 0.001 | 1.2±0.4 | 1.9±0.3 | 0.65 |
| Feeding tube (33)      | 1.0±0.2 | 1.1±0.3 | 1.0±0.0 | 0.059 | 1.3±0.5 | 1.1±0.3 | 0.29 |
| Weight (34-35)         | 1.0±0.1 | 1.1±0.2 | 1.0±0.0 | <0.001 | 1.5±0.0 | 1.5±0.0 | 0.56 |
Table 3. EORTC/RTOG radiation induced toxicity for group A and C.

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<th>Group A</th>
<th>Group C</th>
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<td><strong>Acute toxicity</strong></td>
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<tr>
<td>Grade II</td>
<td>2/11 (18.2%)</td>
<td>1/11 (9%)</td>
<td>0.44*</td>
</tr>
<tr>
<td>Grade III</td>
<td>9/11 (81.8%)</td>
<td>10/11 (91%)</td>
<td></td>
</tr>
<tr>
<td><strong>Late toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>4/11 (36.4%)</td>
<td>5/11 (45.5%)</td>
<td>0.85*</td>
</tr>
<tr>
<td>Grade II</td>
<td>7/11 (63.6%)</td>
<td>6/11 (54.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* Chi² test
Randomization

H&N Mass
- FNA
- Flow Cytometry
- Cell cycle analysis

Conventional
- 70Gy/2Gy/.fr/5days/week
- EORTCQLQ-H&N35

Accelerated
- 64.4Gy/2.3Gy/fr/5days/week
- EORTCQLQ-H&N35

Clinical Response of Residual disease
- FNA
- Flow Cytometry
- Cell cycle analysis
- EORTCQLQ-H&N35
- Toxicity