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ORIGINAL ARTICLE

The role of epidermal growth factor receptor and E-cadherin for the outcome of reduction in the overall treatment time of radiotherapy of supraglottic larynx squamous cell carcinoma

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Abstract

Reduction of the overall treatment time (OTT) of radiotherapy results in increased T-site control in squamous cell carcinomas of the head and neck (HNSCC). However, the response is heterogeneous and accelerated repopulation of clonogenic tumour cells during therapy may be one of the factors determining this response. The aim of the present study was to identify the influence of the epidermal growth factor receptor (EGFr) and E-cadherin for T-site control when the OTT was reduced and whether the markers add information to the histopathological grading in selecting patients for accelerated radiotherapy. A total of 209 patients from randomized DAHANCA-trials with supraglottic larynx squamous cell carcinomas treated with primary radiotherapy with different OTT of 9½, 6½, and 5½ weeks. Available formalin-fixed paraffin embedded tumour tissues were re-evaluated for histopathological characteristics and stained for EGFr and E-cadherin. Data were correlated with patient and tumour characteristics and 5-year T-site control. EGFr and E-cadherin were not associated with patient or tumour characteristics except that EGFr correlated to carcinomas with a well to moderate histopathological feature. Tumours with high EGFr or low E-cadherin did benefit from reduced OTT, and the combination of the two (high EGFr and low E-cadherin) had the most significant acceleration of treatment effect, compared with tumours with other combinations of EGFr and E-cadherin expression. Tumours with high expression of EGFr and low expression of E-cadherin showed the most significant increase in T-site control when the overall treatment time of radiotherapy was reduced, and the markers may be useful for selecting patients who will benefit from accelerated radiotherapy.

The aim of radiotherapy is to destroy clonogenic tumour cells present at the start of treatment as well as those produced during treatment. Some squamous cell carcinomas of the head and neck (HSNCC) have shown the ability to accelerate the production of clonogenic cells during radiotherapy—also called accelerated repopulation—possibly trigged by the injury produced by the ionizing radiation [1]. Consequently, a significant amount of a given radiation dose may be used to sterilize cells produced during treatment and a reduction in the overall treatment time (OTT) means that fewer cells will have to be destroyed.

This principle has been demonstrated in the past where prolongation of OTT has yielded poor results regarding T-site control [2,3]. It was therefore obvious to attempt to minimize the time available for accelerated repopulation between the single fractions of radiotherapy simply by reducing the overall treatment time and keeping the same total dose and dose per fraction. This principle has lately shown the same beneficial trend in several large clinical studies [4,5]. Among these is the Danish DAHANCA 6 & 7 study, which compared 5 fx/wk with 6 fx/wk. It showed an improved 5-year T-site control from 60% to 70% by decreasing the OTT from 6½ week to 5½ week [6] and other studies have shown the same trend [4,5]. However, the price to pay was increased early morbidity [7] and in the case of more than 6 fx/wk, as in the Polish CAIR study, also increased late morbidity [8].

The goal for the future is to tailor the treatment to each patient and tumour type and one way of doing this is to identify the carcinomas capable of accelerated repopulation.

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The experiences from the Danish DAHANCA studies have suggested that the ability to initiate rapid repopulation of clonogenic tumour cells take place at the T-site but not in nodal metastases [9] and appears to be related to well- to moderately differentiated tumours and only to a lesser degree poorly differentiated carcinomas [9,10]. As a consequence, it would be logical to apply accelerated radiotherapy only to well- to moderately differentiated HNSCC, leaving conventional treatment for poorly differentiated head and neck tumours. However, one of the problems is that tumour differentiation is a rather imprecise measure based on morphology and therefore depends very much on the eyes that visualize. To overcome this, many different scoring systems have been elaborated in order to characterize the histopathological grading in a more quantitative way—some focused on the invasive zone [11], others on characteristics applied to all tumour tissue [12]. Consequently, it is difficult to compare gradings from different institutions and sometimes even between pathologists within the same department [13,14].

The question thus arises: Which factors characterize well- to moderately differentiated squamous cell carcinomas of the head and neck and can the expression of different markers replace or add information to the histopathological grading in the selection of patients for accelerated radiotherapy?

The epidermal growth factor receptor (EGFr) is overexpressed in the majority of squamous cell carcinomas [15] and activation of the receptor leads to phosphorylation of the tyrosine kinase domains on the intracellular part of the receptor, activating downstream cascades resulting in altered gene activation and modulation of the cell products. This has been related to increased cell proliferation, decreased apoptotic activity, increased angiogenesis, increased invasive and metastatic potential, and hence increased resistance to anti-tumour therapy [16–18]. Recently, presence of the EGF receptor was shown to be an independent negative predictive factor for squamous cell carcinomas of the head and neck treated with conventional radiotherapy with 5 fx/wk [19]. Conversely, it has been suggested that the prognostic value was related to the OTT, with high EGFr as a negative prognostic factor, when OTT was prolonged and a positive factor when treatment time was accelerated [20]. A similar pattern was seen in a series of 803 patients from the DAHANCA 6 & 7 trial. Tumours with high expression of EGFr did better regarding locoregional control when treated with accelerated radiotherapy whereas there was no benefit of acceleration in tumours with low EGFr. This pattern was even more marked in tumours with high EGFr and well to moderate tumour differentiation compared with tumours with low EGFr and/or poor differentiation (Eriksen JG et al., unpublished observation), suggesting that other parameters were needed to describe the impact of tumour differentiation and OTT on local and locoregional control in squamous cell carcinomas of the head and neck.

E-cadherin is another protein with possible importance for the outcome after radiotherapy. It has previously been suggested that accelerated repopulation was closely related to a decrease in cell density of clonogenic cells and one possible mechanism of initiation of rapid repopulation could be changes in the communication between cells by regulation of E-cadherin and connexins [21,22]. It was also hypothesized that this communication was dependent on an extracellular environment that was identical with the environment from which the malignant cell originates—i.e. the T-site position. Furthermore this was probably only possible in malignant cells which have preserved the functions of communication of normal epithelial cells, as in the well- to moderately differentiated tumours [1]. E-cadherin, a calcium-dependent membrane protein essential for the formation of adherens junctions between cells [23], seemed to fulfil these requirements. It is a marker of differentiation and is often lost in squamous cell carcinomas—especially in poorly differentiated tumours and in lymph node metastases [24–26]. Downregulation of E-cadherin reduces cell–cell adhesion, gap–junction mediated communication [22], and prevents terminal differentiation of cells—thus maintaining the ability of continued proliferation [27]. Hence, loss of E-cadherin seems to be an important step in malignant progression and coincides with the transition from hyperplasic pre-malignant tissue to invasive carcinomas [28].

The aim of the present study was to identify the influence of EGFr and E-cadherin for T-site control when the overall treatment time of radiotherapy was reduced. The second aim was to evaluate whether the expression of E-cadherin and EGFr could add information to the histopathological grading for selecting patients for accelerated radiotherapy.

Material and methods

Patients and treatment

A total of 209 patients with available formalin fixed paraffin embedded tissue blocks of supraglottic larynx squamous cell carcinomas were included in the study. All patients were treated with primary radiotherapy after enrolment in the randomized DAHANCA trials 2, 5, or 6 & 7 receiving 66–68 Gy in fractions of 2 Gy but with different OTT

depending on the schedule of the trial: 35 patients received treatment in a split-course setting over 9½ weeks, 109 patients were treated with conventional radiotherapy over 6½ weeks, whereas the OTT was 5½ weeks for 65 patients treated with an accelerated schedule. All patients were concomitantly treated with a hypoxic radiosensitizer—either misonidazole or nimorazole according to the DAHANCA guidelines. Patient characteristics are given in Table I.

Histopathological grading of tumours

Tumour tissue in the current study was processed at more than 20 different institutions over a period of more than 17 years. In order to overcome differences in histopathological grading, all tumours were re-graded, using a fresh cut haematoxylin-eosin stained slide. The histopathological grading was done using the system developed by Jacobson in 1973 and modified by Lund et al. [29]. The advantage of this system was that it has a more specific description of each parameter and a histological score defined as the total sum of points divided by the number of parameters evaluated and converted to poor or well to moderate tumour differentiation [12,30].

Immunohistochemistry

The tissue sections were deparaffinized in petroleum and alcohol by standard procedures. For the visualization of E-cadherin, slides were boiled in TEG buffer, pH 9.0, in a microwave oven for 3×5 minutes, with refilling of buffer whenever needed. This was followed by a 20-minute block of peroxidase activity in hydrogen peroxide 0.3%. A monoclonal antibody against E-cadherin (clone HECD-1, Zymed, 1:600) was diluted in Antibody Diluent (DAKO) and applied to the tissue sections overnight at 4° celsius. Envision+ (DAKO) was added for 30 minutes and the reaction was visualized using DAB (chromogen and diaminobenzidine) from DAKO. Counterstaining was performed using Mayer's haematoxylin and mounting was done with DPX (BDH Laboratory supplies). Intervening washes were done in Tris/PBS buffer (pH 7.6). Omission of the primary antibody served as negative control and a very well-differentiated squamous cell carcinoma and normal epithelial tissues were used as positive controls. The EGFr detection system DAKO EGFR pharmDx® kit (Clone 2-18C9) was used to visualize the expression of the external part of the EGF receptor. A detailed description of the procedure can be found in the study by Eriksen et al. [20].

The presence of the two receptors was evaluated as the fraction of expression in the tumour/normal tissue borders in the biopsy. The estimated fraction was divided into high or low expression, i.e. more or less than 50% positive stained tumour cells, and only membrane staining was considered. This procedure

Table I. Patient and tumour characteristics and outcome data organized by the overall treatment time of radiotherapy.

Patient and tumour characteristics	9½ week split course	6½ week conventional	5½ week accelerated	Difference
Number of patients	35	109	65	
Median age (years)	62 (47–75)	61 (35–82)	62 (40–81)	n.s.
Female	7 (20%)	28 (26%)	19 (29%)	n.s.
TNM classification				n.s.
T1	1 (3%)	7 (6%)	4 (6%)	
T2	10 (29%)	38 (35%)	28 (43%)	
T3	16 (46%)	37 (34%)	20 (31%)	
T4	8 (23%)	25 (25%)	13 (20%)	
Node positive at diagnosis				n.s.
N+	12 (34%)	36 (33%)	25 (38%)	
N0	23 (66%)	73 (67%)	40 (62%)	
Tumour stage				n.s.
Stage I	0	0	0	
Stage II	9 (26%)	32 (29%)	18 (28%)	
Stage III	16 (46%)	37 (34%)	25 (38%)	
Stage IV	10 (29%)	40 (37%)	22 (34%)	
Histopathological grading				n.s.
Well differentiation	13 (37%)	27 (25%)	22 (34%)	
Moderate differentiation	10 (29%)	44 (40%)	21 (32%)	
Poor differentiation	12 (34%)	38 (35%)	22 (34%)	
T-site recurrence	26 (74%)	45 (41%)	16 (25%)	$p < 0.0001$
Locoregional recurrence	27 (77%)	51 (47%)	23 (35%)	$p < 0.0001$
Disease specific death	21 (60%)	37 (34%)	15 (23%)	$p = 0.004$

was chosen since the most marked loss or gain in expression was seen in the tumour/normal tissue borders compared with more central parts of the biopsy. Intra- and inter-observer variation studies have earlier been performed for these two markers with acceptable results.

Statistics

Statistical evaluation was done using descriptive statistics, Pearson's χ^2 test and Spearman's test for trend. Univariate survival analyses were performed by the Kaplan–Meier method and compared by the log-rank test, using linear trend for factor levels. Multivariate analysis was done using backward Cox proportional hazards analysis. Results were considered significant at levels less than 5% (two-sided tests). Relative risks (RR) were presented with 95% confidence intervals. The endpoint considered was T-site control at 5 years. SPSS 11.0 software was used for the analyses.

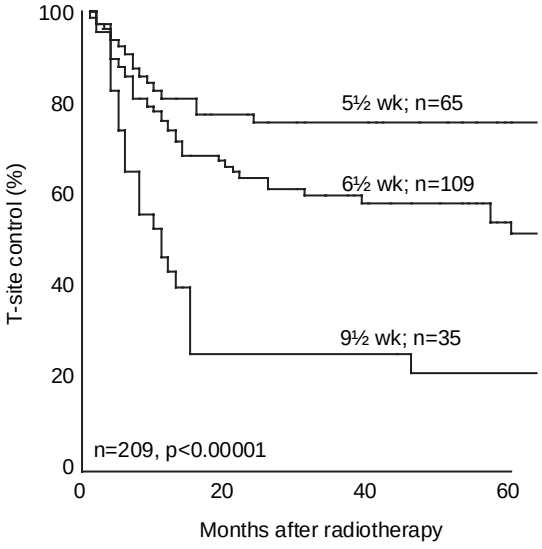

Figure 1. The effect of reduction in overall treatment time with T-site control as endpoint in 209 supraglottic larynx carcinomas.

Results

The patients in the three OTT groups were comparable regarding age, sex, tumour size, presence of nodal metastases at the time of diagnosis, stage according to the TNM system, and histopathological grading but differed markedly regarding T-site control and disease specific survival (see Table I). As the OTT were reduced, the T-site control was improved.

Table II. Correlation of EGFr, E-cadherin and histopathological grading to patient and tumour characteristics and outcome parameters.

	EGFr		E-cadherin		Histopathological grading			Spearman
	Low	High	Low	High	Poor	Moderate	Well	*p*-value
Sex								n.s.
Female	17 (32%)	37 (68%)	28 (52%)*	26 (48%)*	20 (37%)	15 (28%)	19 (35%)	
Male	49 (32%)	106 (68%)	103 (67%)*	51 (33%)*	52 (33%)	60 (39%)	43 (28%)	
T classification								n.s
T1	2 (17%)	10 (83%)	7 (58%)	5 (42%)	7 (58%)	2 (25%)	2 (17%)	
T2	23 (30%)	53 (70%)	47 (62%)	29 (38%)	24 (32%)	22 (29%)	30 (39%)	
T3	25 (34%)	48 (66%)	44 (60%)	29 (40%)	23 (32%)	30 (41%)	20 (27%)	
T4	16 (33%)	32 (67%)	33 (70%)	14 (30%)	18 (37%)	20 (42%)	10 (21%)	
Nodal status								*p = 0.009
N+	26 (36%)	47 (64%)	47 (65%)	25 (35%)	32 (44%)*	27 (37%)*	14 (19%)*	
N0	40 (29%)	96 (71%)	84 (62%)	52 (38%)	40 (30%)*	48 (35%)*	48 (35%)*	
Stage of disease								*p = 0.008
Stage 2	15 (25%)	44 (75%)	38 (64%)	21 (36%)	17 (29%)*	16 (27%)*	26 (44%)*	
Stage 3	25 (32%)	53 (68%)	43 (55%)	35 (45%)	25 (32%)*	31 (40%)*	22 (28%)*	
Stage 4	26 (36%)	46 (64%)	50 (70%)	21 (30%)	30 (42%)*	28 (39%)*	14 (19%)*	
Treatment time								n.s.
9½ week	14 (40%)	21 (60%)	30 (86%)	5 (14%)	12 (34%)	10 (29%)	13 (37%)	
6½ week	36 (33%)	73 (67%)	58 (54%)	50 (46%)	38 (35%)	44 (40%)	27 (25%)	
5½ week	16 (25%)	49 (75%)	43 (66%)	22 (34%)	22 (34%)	21 (32%)	22 (34%)	
T–site recurrence	27 (31%)	60 (69%)	62 (72%)*	24 (28%)*	24 (28%)[1]	32 (37%)[1]	31 (35%)[1]	*p = 0.02; [1]p = 0.05
Locoreg. recurr.	32 (32%)	69 (68%)	73 (73%)*	27 (27%)*	27 (26%)[1]	37 (37%)[1]	37 (37%)[1]	**p = 0.004; [11]p = 0.01
Dead of disease	27 (41%)	46 (32%)	52 (40%)	20 (26%)	27 (37%)	28 (38%)	18 (25%)	n.s.

*indicate correlation between T-site recurrence and E-cadherin.
[1]indicate correlation between T-site recurrence and histopathol. grading.
**indicate correlation between locoreg recurrence and E-cadherin.
[11]indicate correlation between locoreg recurrence and histopathol. grading.

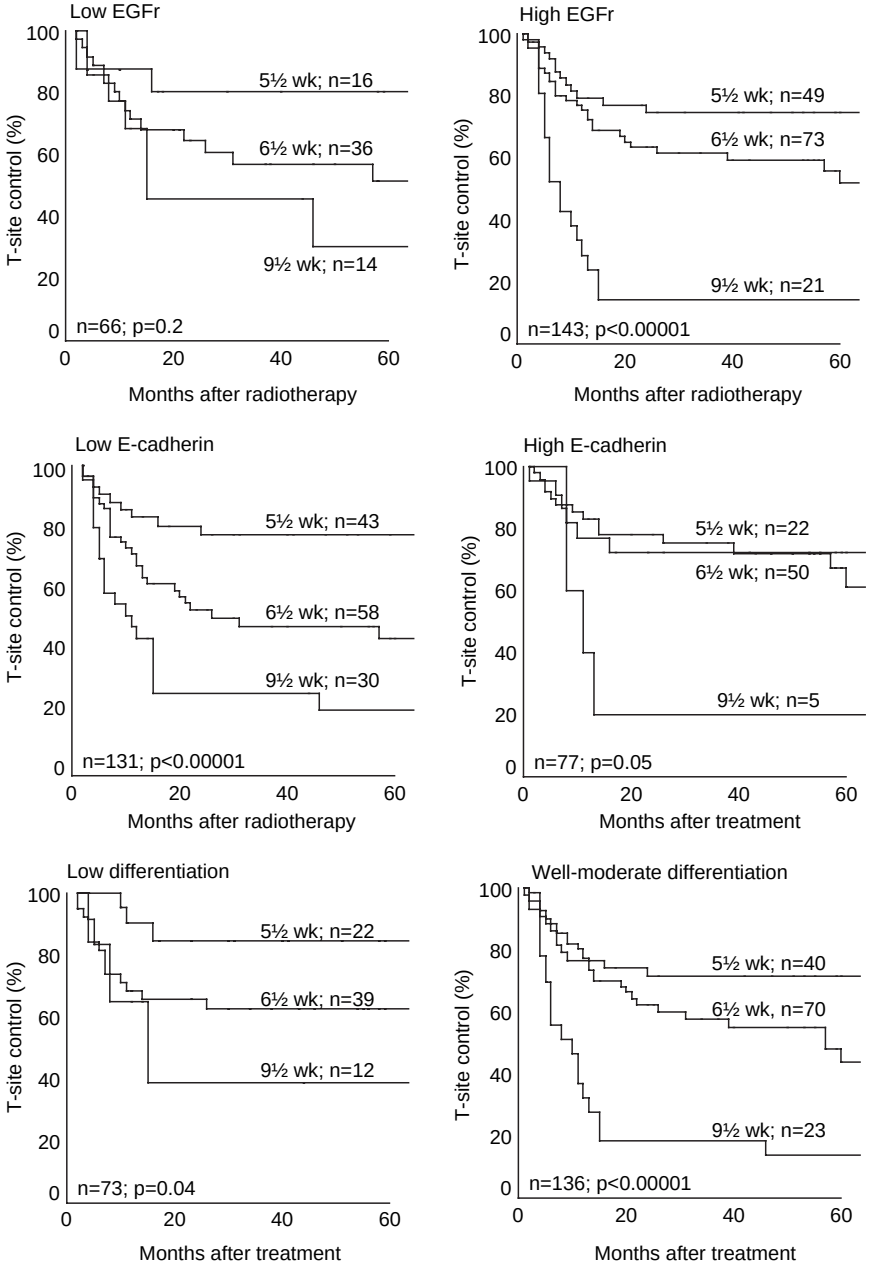

Figure 2. Influence of EGFr, E-cadherin and histopathological differentiation on overall treatment time with T-site control as endpoint.

The overall impact of reduced OTT on T-site control in this study is illustrated in Figure 1 and was comparable to what was seen in the larger trials from which these patients originate [9].

The stainings for EGFr and E-cadherin (dichotomized into high and low expression) were correlated with the clinical and pathological parameters from Table I. The results are given in Table II: generally, no correlation was found between EGFr or E-cadherin and tumour or patient characteristics. However, poor histopathological appearance was associated with the presence of nodal metastases and hence also high tumour stage. EGFr was correlated to well- to moderately differentiated carcinomas ($p = 0.002$), whereas E-cadherin was not ($p = 0.5$). The correlation between high EGFr and low E-cadherin was weak ($p = 0.05$).

The influence of EGFr, E-cadherin and histopathological pattern was correlated with T-site control, using actuarial 5-year survival rates (Figure 2). Data showed that tumours with high expression of EGFr or low expression of E-cadherin seemed to benefit more from a reduction in OTT from 6½ to 5½ weeks compared with tumours with low EGFr or high E-cadherin but the pattern was not unambiguous since the number of tumours in each treatment

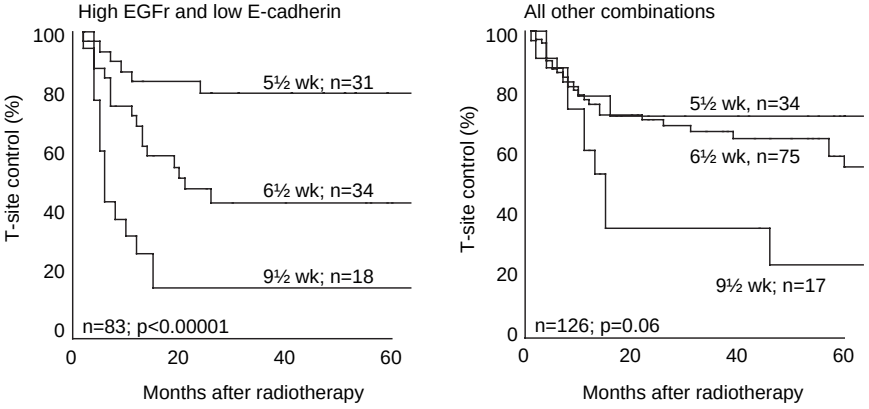

Figure 3. Influence of high EGFr and low E-cadherin or other combinations of EGF/E-cadherin expression on the overall treatment time with T-site control as endpoint.

group was rather small. In this series of patients, T-site control was improved when OTT was reduced regardless of histopathological differentiation.

The benefit of a reduction in OTT was suggested to be a feature of tumours with high expression of EGFr or low expression of E-cadherin, and data from the literature indicate that EGFr down-regulate E-cadherin [31]. Therefore we evaluated the response of a reduction in OTT for tumours with high EGFr and low E-cadherin using 5-year actuarial T-site control as endpoint and compared it with all other combinations of EGFr and E-cadherin expression (Figure 3). Data suggested that T-site control was improved after reduction in OTT from 6½ to 5½ weeks in tumours with high EGFr and low E-cadherin expression whereas it was not the case for tumours with other combinations of EGFr and E-cadherin expression. Tumours treated with very long OTT of 9½ weeks showed poor T-site control independent of expression of these markers.

In a Cox proportional hazard analysis (Table III), there was no difference in the relative risk for T-site failure whether tumours had a high or low histopathological score—i.e. reduction in OTT was an independent parameter for T-site failure in both groups ($p = 0.02$ and $p < 0.0001$ respectively). On the contrary, only low E-cadherin ($p < 0.0001$) or high EGFr ($p < 0.0001$) were significant markers for T-site failure after radiotherapy and RR seemed to be accentuated in the tumours containing the combined pattern ($p < 0.0001$). However, carcinomas with other combinations of EGFr and E-cadherin expression were also significant ($p = 0.04$). When these combinations were split up, as seen in Table IV, only tumours with high EGFr and low E-cadherin did benefit from a reduction in OTT ($p < 0.0001$).

The influence of the histopathological score on the prognostic value of the markers was further explored and data suggested that the combination of high EGFr and low E-cadherin still contained prognostic information regarding OTT and T-site failure, whether the tumour morphology had a low or high histopathological differentiation RR 4.5 (2.3–8.8) and 7.2 (1.2–42.7), respectively. This was not the case in other combinations regardless of low or high histopathological differentiation RR 2.0 (0.7–5.2) and 2.0 (0.9–4.3). However, the differences in RR do suggest that other parameters as well may be important for the selection of HNSCC patients for radiotherapy with accelerated schedules.

Discussion and conclusion

The 209 patients in the current study were selected from three different randomized trials on the basis of available tumour tissues, site defined as supraglottic larynx and concomitant treatment with a hypoxic sensitizer (misonidazole or nimorazole). The three different OTT groups were not different from each other regarding tumour and patient characteristics, but differed significantly regarding T-site control after radiotherapy in a pattern comparable with all patients from the trials where they originate. Nevertheless, the effect of reduced OTT on T-site control when poorly differentiated tumours were separated from the well- to moderately differentiated carcinomas were not similar to the total patient cohort where improved T-site control of reduced OTT was observed in the well- to moderately differentiated carcinomas [9]. This has to be kept in mind when extrapolating data from this subgroup of patients. The reason for this discrepancy could be that the impact of tumour cell differentiation was less marked in supraglottic larynx carcinomas compared with other sites and, furthermore, we had systematically regraded all tumours after the modified Jacobson system, which has changed the status of 14% of the carcinomas.

Table III. Cox regression analysis, showing hazard ratios with local T-site failure as endpoint. The data-set was divided by histopathological grading, E-cadherin, EGFr, or the combination of the two parameters (see also Table IV for details).

	Poor diff. RR (95%C.I)	Well–mod.diff. RR (95%C.I)	Low E–cadherin RR (95%C.I)	High E-cadherin RR (95%C.I)	High EGFr RR (95%C.I)	Low EGFr RR (95%C.I)	High EGFr and low E-cadherin RR (95%C.I)	Other combinations RR (95%C.I)
Number of patients	73	136	131	77	143	66	83	126
T-stage (T1–2 vs. T3–4)	0.9 (0.4–2.2)	1.8 (1.1–3.1)	1.5 (0.9–2.6)	1.2 (0.5–2.9)	**1.8 (1.1–3.1)**	0.8 (0.4–2.0)	**2.3 (1.1–4.4)**	1.1 (0.6–2.0)
N-stage (N0 vs. N+)	1.2 (0.5–2.8)	1.0 (0.6–1.8)	0.9 (0.5–1.6)	1.5 (0.6–3.4)	1.2 (0.7–2.0)	1.1 (0.5–2.4)	0.8 (0.4–1.7)	1.3 (0.7–2.3)
Sex (male vs. female)	0.6 (0.2–1.6)	0.7 (0.4–1.4)	0.6 (0.3–1.3)	1.2 (0.5–2.9)	**0.5 (0.2–1.0)**	1.1 (0.4–2.6)	0.6 (0.2–1.6)	0.7 (0.3–1.3)
Overall treatment time	**2.4 (1.1–5.2)**	**2.9 (1.8–4.6)**	**2.5 (1.6–3.9)**	1.5 (0.6–3.4)	**3.3 (2.1–5.3)**	2.0 (0.9–4.2)	**3.7 (2.2–6.5)**	**1.9 (1.0–3.4)**

Bold indicate significant relations.

There was no correlation of EGFr and E-cadherin to clinical and tumour characteristics (see Table II), except that EGFr was correlated to well- to moderately differentiated carcinomas. This has also been reported earlier [20] as well as the opposite has been observed [32]. No association was found in the present study between the expression of E-cadherin and well- to moderately differentiated carcinomas, which was surprising, since several earlier studies have found E-cadherin to be a marker of differentiated squamous cell carcinomas [30,33]. Perhaps this was related to differences in tumour grading and biology or evaluation of the E-cadherin staining, since no consensus exists.

A reduction in OTT of radiotherapy is important in order to decrease the cell proliferation between fractions of radiotherapy. Several observations have suggested that high levels of EGFr and activation of the EGF system increase cell proliferation as well as inhibiting apoptosis [34,35]. Furthermore, reductions in cell–cell adhesion via a reduction in E-cadherin promote the change in phenotype from pre-malignant epithelial tissue to invasive squamous cell carcinoma [28]. Loss of E-cadherin decreases adhesion and enhances metastatic behaviour but also inhibits terminal differentiation by maintaining high levels of β1 integrins and thereby preserves the capability of proliferation [27]. Not only do high levels of EGFr and low levels of E-cadherin maintain the ability of cell proliferation but it was also recently suggested that EGF downregulates E-cadherin, [36] and in addition the combination of high EGFr and low E-cadherin was suggested to be responsible for poor survival [37,38].

In our study, univariate analyses (see Figure 2) showed that tumours with high expression of EGFr or low expression of E-cadherin had a significant benefit of reduction in OTT when T-site control was used as endpoint. For the combined parameters (see Figure 3), the difference was primarily seen in the reduction from 6½ to 5½ weeks of OTT, whereas no or little difference was observed in the patients treated with 9½ weeks' OTT. This is possibly because there was a very long time for repopulation – even for slowly proliferating tumours – but the number of patients in the 9½ week arm was also relative low (in total: n = 35) and there might be a risk of a type 2 error.

A combined analysis of high EGFr and low E-cadherin showed a more pronounced separation between the three OTT arms compared with other combinations (see Figure 3) and this was confirmed in a multivariate Cox regression analysis (see Table III), where a reduction in OTT was found to be important for T-site failure in tumours expressing high levels of EGFr or low E-cadherin. The RR

Table IV. Cox regression analysis, with local T-site failure as endpoint, showing the four combinations of EGFr and E-cadherin expression. 'High EGFr and high E-cadherin', 'Low EGFr and low E-cadherin', and 'Low EGFr and high E-cadherin' are identical with 'other combinations' in Table III.

	High EGFr and low E-cadherin RR (95% CI)	High EGFr and high E-cadherin RR (95% CI)	Low EGFr low E-cadherin RR (95% CI)	Low EGFr high E-cadherin RR (95% CI)
Number of patients	83	59	48	18
T-stage (T1–2 vs. T3–4)	**2.3 (1.1–4.4)**	1.2 (0.5–3.2)	0.9 (0.4–2.1)	1.4 (0.1–16.5)
N-stage (N0 vs. N+)	0.8 (0.4–1.7)	1.7 (0.6–4.6)	1.0 (0.4–2.7)	1.4 (0.1–14.1)
Sex (male vs. female)	0.6 (0.2–1.6)	0.4 (0.1–1.2)	0.7 (0.2–2.3)	3.4 (0.5–24.3)
Overall treatment time	**3.7 (2.2–6.5)**	2.6 (0.8–8.1)	1.6 (0.7–3.6)	3.4 (0.3–42.0)

Bold indicate significant relations.

was higher for the combination of EGFr and E-cadherin than for EGFr or E-cadherin alone, but the 95% confidence intervals overlapped. Also, the group of other combinations of EGFr and E-cadherin expression was significant in the multivariate analysis but this significance disappeared when the different combinations were analysed separately (see Table IV).

The univariate and multivariate analyses separating the study group by histopathological feature indicated, no difference in T-site control when OTT was reduced and a further analysis of the combination of high EGFr and low E-cadherin and histopathological differentiation suggested that the marker combination high EGFr and low E-cadherin still contained prognostic information regarding OTT and T-site failure, whether the tumour morphology had a low or high histopathological differentiation, RR 4.5 (2.3–8.8) and 7.2 (1.2–42.7) respectively, whereas it was not the case in other combinations regardless of low or high histopathological differentiation status, RR 2.0 (0.7–5.2) and 2.0 (0.9–4.3), respectively. However, the differences in RR do suggest that other parameters as well may be important for the selection of HNSCC patients for radiotherapy with accelerated schedules.

We conclude that high EGFr and low E-cadherin in combination seemed able to separate patients who responded with increased T-site control when OTT was reduced. Probably, these two markers alone do not carry enough information for a precise identification of tumours capable of accelerated repopulation.

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