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EGFR and Phosphorylated EGFR in Relation to HPV and Clinical Outcome in Tonsillar Cancer

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Abstract. *Background: Human papillomavirus (HPV) is a risk factor for tonsillar squamous cell carcinoma (TSCC) and the presence of HPV is correlated to a better clinical outcome. To find additional biomarkers that, together with HPV, predict clinical outcome, the aim of the present study was to evaluate epidermal growth factor receptor (EGFR) and phosphorylated EGFR (pEGFR) in relation to HPV status and clinical outcome. Materials and Methods: A total of 83 pre-treatment TSCC biopsies were analyzed for EGFR and pEGFR Tyr1068 and Tyr1148 by immunohistochemistry, and the obtained data were tested for correlation to tumor HPV status and disease-free survival. Results: The presence of pEGFR Tyr1068 and 1148, both correlated significantly to the absence of HPV. However, neither of these, nor total EGFR, correlated significantly to disease-free survival for HPV-positive or HPV-negative TSCC. Conclusion: Since pEGFR Tyr1068 and 1148 are correlated to absence of HPV but not to clinical outcome, these may not be optimal prognostic markers for clinical outcome in patients with TSCC.*

During the last decade, human papillomaviruses (HPV) have become established as a major risk factor for the development of tonsillar squamous cell carcinoma (TSCC) (1). In addition, several studies from both Europe and the US have reported an increase in the incidence of TSCC, with HPV indicated as the cause of this increase (2, 3). At present, 50-90% of all oropharyngeal squamous cell carcinomas (OSCC) are reported to be HPV-positive (HPV^+) (4). It has also been recognized that HPV^+ and HPV-negative (HPV^-) TSCCs should be regarded as separate diseases, differing in several important characteristics (4). An important difference is in prognosis, where patients with HPV^+ TSCC have a markedly better survival than patients with HPV^- TSCC, at approximately 80% and 40% 5-year survival respectively, and the survival for patients with HPV^- TSCC is in line with the rate for patients with head and neck squamous cell carcinoma (HNSCC) in general (5-7).

In recent years, due to low overall survival, the oncological treatment of patients with HNSCC has become intensified, and may now include a combination of induction chemotherapy, and/or combined chemoradiotherapy, hyperfractionated radiotherapy (RT) and epidermal growth factor receptor (EGFR) inhibitors. A drawback of this intensified treatment is that it also causes an increase in the number and intensity of both acute and chronic side-effects. Earlier studies from our group show that the majority of patients with HPV^+ TSCC are recurrence-free after conventional radiotherapy-alone, indicating that intensified treatment may be unnecessary, and even adverse, for many of these patients (6). There is, thus, a need to identify biomarkers that, together with HPV, can be used to identify patients that need or do not need intensified treatment.

EGFR is frequently overexpressed in various types of epithelial tumors, including HNSCC (8, 9). Amplification of the *EGFR* gene is common in HNSCCs, while most studies report the frequency of EGFR mutations in HNSCC to be low (10-12). Several studies, but not all, have associated both EGFR overexpression and amplification with worse prognosis in HNSCC (10, 13-15). Importantly, some drugs used in adjuvant therapy are specifically directed towards cells overexpressing EGFR *e.g.* gefitinib and erlotinib (cetuximab) (16). As a consequence, the *EGFR* status of a tumor could be of importance for tumor response to adjuvant therapy.

Activated EGFR is phosphorylated at one or more of several different tyrosine residues, with Tyr1068, Tyr1148 and Tyr1173 as the major sites, leading to the activation of several downstream pathways *e.g.* mitogen-activated protein kinase (MAPK) and Akt (17, 18). The presence of different phosphorylated species of EGFR has also been linked to clinical outcome, both for patients with HNSCC and for those with other tumor types (19, 20).

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Although several studies have been performed on EGFR in HNSCC, including oropharyngeal squamous cell carcinoma, in relation to clinical outcome, the HPV status of the tumors have, in the majority of these studies, not been taken into account (13-14). Since HPV is in itself an important marker for clinical outcome, it is important to evaluate the association between EGFR and clinical outcome for HPV^+ and HPV^- tumors separately, in particular because some reports demonstrate a correlation between tumor HPV status and EGFR overexpression (21).

The purpose of the present pilot study was to examine if the presence of total EGFR and/or EGFR phosphorylated on tyrosine residues 1068 or 1148 can be used as predictive markers for treatment response for TSCC in combination with HPV status of the tumors.

Materials and Methods

Patients and materials. The 83 TSCC samples included in this study were selected from a cohort of 182 patients, diagnosed with TSCC (ICD-10 classification C09.0-9) between 2000-2006, with available pre-treatment paraffin-embedded tumor biopsies, and treated with intent-to-cure at the Karolinska University Hospital, Stockholm, Sweden. The same 83 TSCC samples were also included in an earlier study on tumor-infiltrating T-cells and details of both patients and tumors are presented elsewhere (22). Briefly, the TSCC samples were categorized both by HPV status [HPV^+ tumors defined as both HPV DNA positive and $p16^{INK4A}$-positive ($p16^+$)] and clinical outcome, where good clinical outcome was defined as 3-year progression-free survival (no relapse and alive three years after diagnosis) and poor clinical outcome was defined as relapsed of disease and/or dead of disease within three years after diagnosis. These 83 samples included all (n=31) HPV^- $p16^{INK4A}$-negative ($p16^-$) samples from the cohort and all patients (n=21) with HPV^+, $p16^+$ tumors with a poor clinical outcome and a random sample (n=31) of all patients (n=109) with HPV^+ $p16^+$ tumors with a good clinical outcome. A sample of 31 out of the 109 original tumors in this category was chosen, since this group dominated, comprising of 68% of the total number of tumors. Data on the presence of high-risk HPV types [presence of HPV DNA assayed by polymerase chain reaction (PCR)] and p16INK4A status (by immunohistochemistry) were obtained from previous studies (3, 6, 23). $p16^+$ TSCC was defined by the presence of >75% $p16^+$ tumor cells. The HPV type for all HPV^+ TSCC was $HPV16^+$ with the exception of two tumors, one with HPV33 and one with HPV56.

Treatment for 79 of the patients was accelerated RT (1.1+2.0 Gy/day for 4.5 weeks, total dose 68 Gy) or conventional RT (2.0 Gy/day, for 6.5-7 weeks, total dose 68 Gy). Four patients had induction chemotherapy followed by concomitant RT (CRT). The study was approved by the Ethical Committee at the Karolinska Institute, Stockholm, Sweden, according to the ethical permissions 2005/431-31/4, 2005/1330-32 and 2009/1278-31/4.

Immunohistochemistry. Formalin-fixed, paraffin-embedded sections of 4 μm, were stained with the rabbit monoclonal antibodies EGFR D38B1, the rabbit polyclonal antibody pEGFR (Tyr1148) or the mouse monoclonal antibody pEGFR (Tyr1068) 1H12, were all used at a dilution of 1:200 and were all from Cell Signaling Technology (Beverly, MA, USA). All sections were subsequently incubated with a biotinylated secondary anti-mouse antibody (1:200, Vector Laboratories, Burlingame, CA, USA) followed by incubation with the avidin-biotin-peroxidase complex using the VECTASTATIN® Elite® ABC kit (Vector Laboratories) and developed with 3, 3'-diaminobenzidine (DAB). The staining was evaluated by two investigators (MR and AN) blinded to tumor HPV status and clinical outcome, and in cases of disagreement, a consensus was made. The fraction of positively-stained cells was evaluated semi-quantitatively in four grades according to the fraction of stained malignant cells: 0, 0%; 1, 1-25%; 2, 26-75%; or 3, 76-100%. The intensity of the staining was also scored separately as: absent, 0; weak, 1; moderate, 2; or strong, 3. For evaluation of the results, the staining was dichotomized as follows: For pEGFR Tyr1068 and 1148, all tumors with positive cells (fraction=1-3 and intensity=1-3) were regarded as positive. For total EGFR, tumors with a staining intensity of 2 or 3 were regarded as positive.

Statistics. Logistic regression models were used to compare categorical data and results are presented as odds ratios with 95% confidence intervals. These analyses were performed in STATA 11 (StataCorp, Texas, USA).

Disease-free survival (DFS) was defined as the time from the date of diagnosis to the date of the last known occasion that the patient was disease-free or to the date of disease recurrence (local, regional or distant). Death without documented recurrence was censored at the date of death. Kaplan–Meier curves were used to present survival data and the log-rank test was used to compare survival curves. These analyses were performed in SPSS (IBM SPSS Statistics, v20, New York, USA).

The associations of immunostaining with TNM classification, stage or histopathological differentiation in Table II were calculated by the Fisher's exact test. Two-sided *p*-values are reported. An independent, two-sided *t*-test was performed to compare the mean age for patients with tumors with positive and negative immunostaining. These calculations were performed in STATA 11.

Results

EGFR in relation to TSCC HPV status. All TSCC samples were stained for total EGFR, and EGFR phosphorylated on residue Tyr1068 or Tyr1148. Examples of immunostaining are presented in Figure 1.

In general, HPV^- TSCC were more often EGFR-positive than HPV^+ TSCC (81 and 71%, respectively), although this difference was not significant (Table I). However, the presence of pEGFR, both for Tyr1068 and Tyr1048 was significantly correlated to the absence of HPV in the tumors, Table I. Thus, 35% and 68% of HPV^- TSCC, as compared to only 12% and 35% of HPV^+ TSCC, were positive for phosphorylation of Tyr1068 and Tyr1148, respectively. Furthermore, more TSCC cases were positive for phosphorylation of Tyr1148 than of Tyr1068, and there was no correlation in the phosphorylation of these two sites.

Correlation between EGFR, pEGFR and patients' and tumor characteristics. The presence of EGFR, pEGFR and Tyr1068 and pEGFR Tyr1148 in relation to patients' and tumor characteristics is presented in Table II. All data were evaluated

Table I. *Immunostaining of epidermal growth factor (EGFR) on tonsillar cancer in relation to tumor human papillomavirus (HPV) status and clinical outcome.*

	Clinical outcome[a]	Staining[b]	HPV status[c]		Unadjusted effects[d]		Effects[d] adjusted for clinical outcome	
			Positive (n=52)	Negative (n=31)	Odds-ratio (95% CI)	*p*-Value[d]	Odds-ratio (95% CI)	*p*-Value[d]
EGFR	Good	Medium/high	23 (74%)	8 (73%)	1			
		Absent/low	8 (26%)	3 (27%)	1.1 (0.23-5.1)	0.92		
	Poor	Medium/high	14 (67%)	17 (85%)	1			
		Absent/low	7 (33%)	3 (15%)	0.35 (0.077-1.62)	0.18		
						0.41[e]		
	Total	Medium/high	37 (71%)	25 (81%)	1		1	
		Absent/low	15 (29%)	6 (19%)	0.60 (0.20-1.73)	0.34	0.59 (0.20-1.77)	0.35
Tyr 1068	Good	Present	2 (6%)	6 (55%)	1			
		Absent	29 (94%)	5 (45%)	0.057 (0.009 -0.37)	0.003		
	Poor	Present	4 (19%)	5 (25%)	1			
		Absent	17 (81%)	15 (75%)	0.71 (0.16-3.12)	0.65		
						0.11[e]		
	Total	Present	6 (12%)	11 (35%)	1		1	
		Absent	46 (88%)	20 (65%)	0.24 (0.077-0.73)	0.012	0.23 (0.071-0.73)	0.013
Tyr 1148	Good	Present	11 (35%)	7 (64%)	1			
		Absent	20 (65%)	4 (36%)	0.31 (.075-1.32)	0.11		
	Poor	Present	7 (33%)	14 (70%)	1			
		Absent	14 (67%)	6 (30%)	0.21 (0.057-0.80)	0.022		
						0.72[e]		
	Total	Present	18 (35%)	21 (68%)	1		1	
		Absent	34 (65%)	10 (32%)	0.25 (0.098-0.65)	0.95	0.25 (0.096-0.67)	0.006

[a]Clinical outcome as defined in the Material and Methods. [b]Intensity of staining. [c]HPV data obtained from previous studies (3, 6, 23). [d]Effects estimated using logistic regression. [e]Overall test for effect modification (*i.e.* interaction between HPV status and clinical outcome).

separately for HPV^+ and HPV^- TSCC. Only one correlation was found to be significant: Staining for phosphorylated Tyr1068 in relation to T-classification for HPV^- TSCC (p=0.023). However, when these tumors were dichotomized and TSCC with classification T1+T2 were compared to those with T3+T4, no significant correlation was found (p=0.45).

Correlation between EGFR, pEGFR and clinical outcome. In order to study the correlation between the presence of EGFR, pEGFR Tyr1068 and pEGFR Tyr1148 and DFS, a Kaplan–Meier analysis was performed, separately for HPV^+ and HPV^- TSCCs (Figure 2). No significant difference was found for any parameter although there was a tendency towards a better outcome for the six patients with HPV^- TSCC and absent or weak EGFR staining (Figure 2B). In addition, no significant difference was found when the immunostaining of EGFR, pEGFR Tyr1068 and pEGFR Tyr1148 was compared for HPV^+ and HPV^- TSCCs between the groups with good and poor clinical outcome as presented in Table III.

Discussion

In the present study we found a significant correlation between the presence of EGFR phosphorylated at residue Tyr1068 or Tyr1148 and absence of HPV in TSCC, but not between EGFR and clinical outcome when patient groups were stratified by HPV status.

EGFR is frequently reported to be overexpressed in HNSCC, although the figures vary between 38-90% of the tumors, possibly due to the different evaluation criteria used (8-9). Our results are comparable to those of Hong *et al*. for EGFR in HPV^+ and HPV^- oropharyngeal tumors (21). They found 78% of HPV^+ and 93% of HPV^- oropharyngeal tumors to be EGFR-positive, similar to our values of 71 and 81%. In contrast to their study, the difference between HPV^+ and HPV^- TSCC was not significant in our study. However, it should be noted that since we used a sample of HPV^+ TSCC from patients with good clinical outcome, with a tendency towards fewer being EGFR-positive, this may have

Table II. *Immunostaining of epidermal growth factor (EGFR) on tonsillar cancer in relation to patients' and tumor characteristics.*

Characteristics	All tumors (n=83)		HPV-positive (n=52)														HPV-negative (n=31)													
							EGFR			Tyr1068				Tyr 1148					EGFR			Tyr1068				Tyr 1148				
			All HPV-positive		Positive (n=43)		Negative (n=9)			Positive (n=6)		Negative (n=46)		Positive (n=18)		Negative (n=34)			All HPV-negative		Positive (n=28)		Negative (n=3)			Positive (n=11)		Negative (n=20)		Positive (n=21)		Negative (n=10)	
	n	%	n	%	n	%	n	%	*p*-Value	n	%	n	%	*p*-Value	n	%	n	%	*p*-Value	n	%	n	%	n	%	*p*-Value	n	%	n	%	*p*-Value	n	%	n	%	*p*-Value
Gender																																				
Male	62	75	36	69	33	77	3	33	0.18	6	100	30	65	0.16	11	61	25	74	0.53	26	84	23	92	3	50	1.00	9	82	17	85	1.00	17	81	9	90	0.65
Female	21	25	16	31	10	23	6	67		0	0	16	35		7	39	9	26		5	16	5	20	0	0		2	18	3	15		4	19	1	10	
TNM																																				
T1	15	18	10	19	8	19	2	22	0.79	1	17	9	20	0.93	4	22	6	18	0.95	5	16	4	16	1	17	0.17	3	27	2	10	0.023	4	19	1	10	0.96
T2	30	36	22	42	17	40	5	56		3	50	19	41		8	44	14	41		8	26	8	32	0	0		0	0	8	40		5	24	3	30	
T3	24	29	14	27	12	28	2	22		1	17	13	28		4	22	10	29		10	32	10	40	0	0		6	55	4	20		7	33	3	30	
T4	15	18	6	12	6	14	0	0		1	17	5	11		2	11	4	12		9	29	7	28	2	33		2	18	6	30		6	29	3	30	
N0	17	20	5	10	5	12	0	0	0.65	1	17	4	9	0.11	2	11	3	9	0.72	12	39	11	44	1	17	1.00	6	55	6	30	0.24	9	43	3	30	0.84
N1	23	28	19	37	12	28	2	22		2	33	17	37		5	28	14	41		4	13	4	16	0	0		0	0	4	20		2	10	2	20	
N2	39	47	27	52	20	47	7	78		2	33	25	54		11	61	16	47		12	39	10	40	2	33		5	45	7	35		8	38	4	40	
N3	4	5	1	2	1	2	0	0		1	17	0	0		0	0	1	3		3	10	3	12	0	0		0	0	3	15		2	10	1	10	
M0	82	99	51	98	42	98	9	100	1.00	5	83	46	100	0.11	17	94	34	100	0.35	31	100	28	112	3	50	1.00	11	100	20	100	1.00	21	100	10	100	1.00
M1	1	1	1	2	1	2	0	0		1	17	0	0		1	6	0	0		0	0	0	0	0	0		0	0	0	0		0	0	0	0	
Stage																																				
I	4	5	0	0	0	0	0	0	0.15	0	0	0	0	0.46	0	0	0	0	0.47	4	13	4	16	0	0	0.79	3	27	1	5	0.27	3	14	1	10	1.00
II	6	7	3	6	1	2	2	22		1	17	2	4		2	11	1	3		3	10	3	12	0	0		0	0	3	15		2	10	1	10	
III	28	34	21	40	18	42	3	33		2	33	19	41		6	33	15	44		7	23	7	28	0	0		2	18	5	25		5	24	2	20	
IV	45	54	28	54	22	51	6	67		3	50	25	54		10	56	18	53		17	55	14	56	3	50		6	55	11	55		11	52	6	60	
Differentiation																																				
Low	49	59	34	65	31	72	3	33	0.055	4	67	30	65	0.086	10	56	24	71	0.34	15	48	15	60	0	0	0.13	5	45	10	50	1.00	8	38	7	70	0.16
Medium	30	36	17	33	11	26	6	67		1	17	16	35		8	44	9	26		13	42	10	40	3	50		5	45	8	40		11	52	2	20	
High	4	5	1	2	1	2	0	0		1	17	0	0		0	0	1	3		3	10	3	12	0	0		1	9	2	10		2	10	1	10	
Age (years)																																				
Mean	60.3		58.6		59.8		53.0		0.078	58.8		58.6		0.95	56.2		59.9		0.24	63.2		63.7		58.3		0.31	66.5		61.4		0.11	63.2		63.9		0.98
Median	60		59		60		51			59		59			55		60.5			61		61		59			62		60.5			61		63		

[a]For EGFR, positive staining is defined as staining with an intensity of 2-3. For Tyr 1068 and Tyr 1148, positive staining is defined as presence of staining. *p*-Values were calculated with the Fischer's exact test.

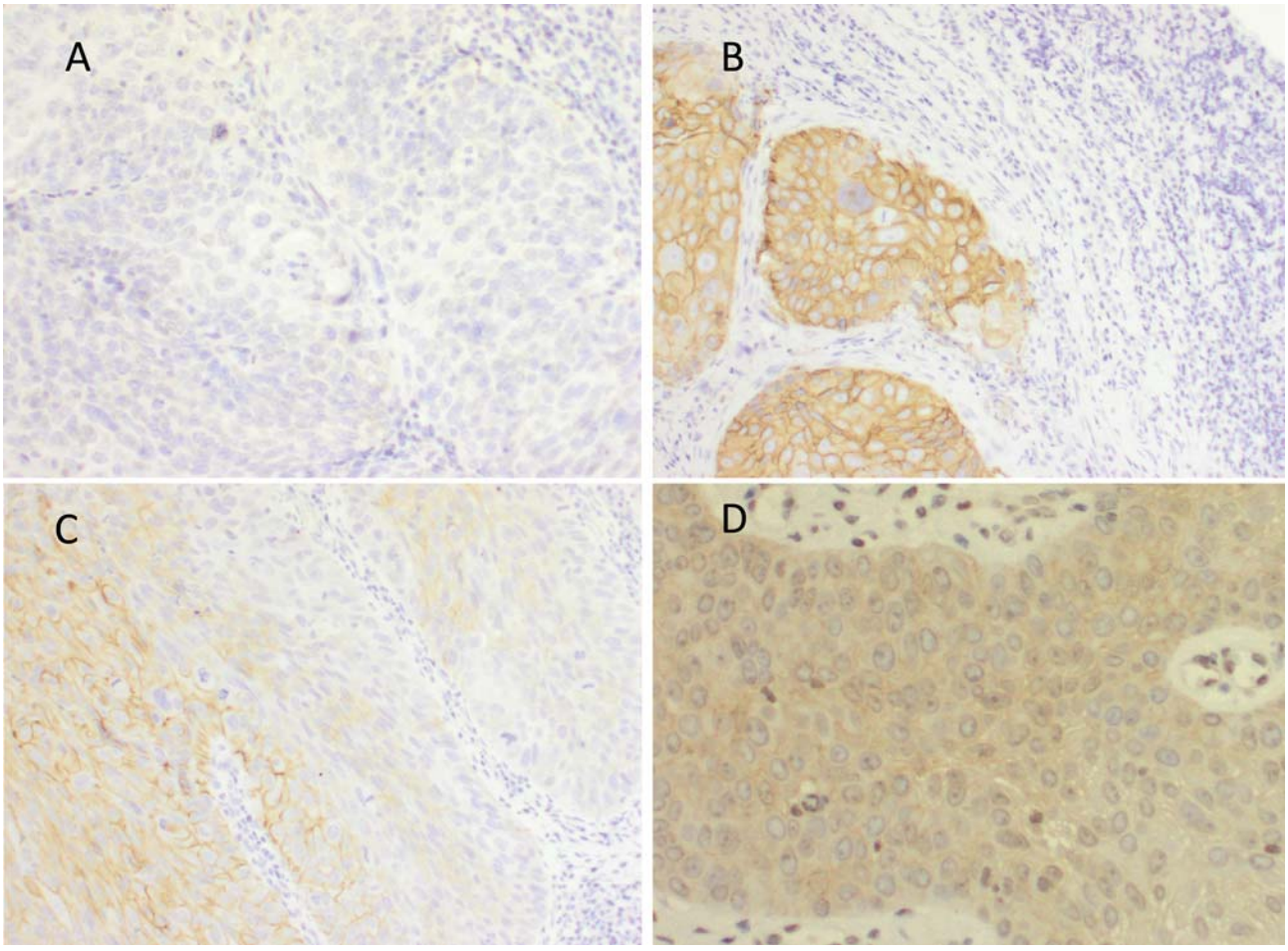

Figure 1. *Epidermal growth factor receptor (EGFR) and phosphorylated EGFR (pEGFR) staining of tonsillar squamous cell carcinoma (TSCC). A: TSCC negative for EGFR staining. B-D: TSCC positive for EGFR (B), pEGFR Tyr1068 (C), and pEGFR Tyr1148 (D). Magnification ×400.*

resulted in a smaller difference in EGFR positivity between HPV^+ and HPV^- cases than there would have been if all HPV^+ TSCC were included.

In line with some other studies (24-26), we did not find any correlation between EGFR and clinical outcome. However, others have found an association between overexpression of EGFR and worse prognosis for HNSCC (13-15, 21, 27-29). Nevertheless, for the few (n=6) patients with HPV^- TSCC with absent/weak EGFR staining, there was a tendency for a correlation with a better clinical outcome.

Although several previous studies have investigated the presence of pEGFR in oropharyngeal and other HNSCC tumors, this has not been correlated with tumor HPV status. We found both EGFR Tyr1068 and Tyr1148 to be significantly correlated to the absence of HPV. This is in line with a study on penile cancer, where HPV^- tumors were more often (40%) pEGFR Tyr845-positive than were HPV^+ tumors (16%) (30). However, since phosphorylation of Tyr845 was not investigated in the present study, a comparison with our study is not clear-cut.

In addition, we did not find a correlation between the presence of EGFR phosphorylated on tyrosine 1068 or 1148 and a worse clinical outcome. This is in line with a study by Aquino *et al*. on pEGFR in OSCC, where no significant correlation between the presence of pEGFR Tyr845 or Tyr1068 and overall survival was found (31). In contrast, some reports do demonstrate a correlation between the presence of pEGFR Tyr1068 and relapse-free survival in HNSCC (19-20). It should be noted that as tumor HPV status was not taken into account in these studies, the results may, at least partly, be due to an indirect effect of pEGFR Tyr1068 being more common in HPV^- HNSCC, while patients with HPV^+ oropharyngeal cancer have a better clinical outcome (6).

We did not find any significant correlation between tumor characteristics such as TNM classification, stage, differentiation and immunostaining, with the exception of T-

Table III. *Immunostaining of epidermal growth factor receptor (EGFR) on tonsillar cancer in relation to clinical outcome and tumor human papillomavirus (HPV) status.*

	HPV status[a]	Staining[b]	Clinical outcome[c]		Unadjusted effects[d]		Effects[d] adjusted for HPV status	
			Good (n=42)	Poor (n=41)	Odds-ratio (95% CI)	*p*-Value[d]	Odds-ratio (95% CI)	*p*-Value[d]
EGFR	Positive	Medium/high	23 (74%)	14 (67%)	1			
		Absent/low	8 (26%)	7 (33%)	1.4 (0.43-4.83)	0.56		
	Negative	Medium/high	8 (73%)	17 (85%)	1			
		Absent/low	3 (27%)	3 (15%)	0.47 (0.077-2.87)	0.41		
						0.18[e]		
	Total	Medium/high	31 (74%)	31 (76%)	1		1	
		Absent/low	11 (26%)	10 (24%)	0.91 (0.34-2.45)	0.85	1.02 (0.367-2.83)	0.97
Tyr 1068	Positive	Present	2 (6%)	4 (19%)	1			
		Absent	29 (94%)	17 (81%)	0.29 (0.048 -1.77)	0.18		
	Negative	Present	6 (55%)	5 (25%)	1			
		Absent	5 (45%)	15 (75%)	3.6 (0.76-17.13)	0.11		
						0.65[e]		
	Total	Present	8 (19%)	9 (22%)	1		1	
		Absent	34 (81%)	32 (78%)	0.84 (0.29-2.43)	0.74	1.20 (0.38-3.80)	0.76
Tyr 1148	Positive	Present	11 (35%)	7 (33%)	1			
		Absent	20 (65%)	14 (67%)	1.1 (0.34-3.54)	0.87		
	Negative	Present	7 (64%)	14 (70%)	1			
		Absent	4 (36%)	6 (30%)	0.75 (0.16-3.58)	0.72		
						0.022[e]		
	Total	Present	18 (43%)	21 (51%)	1		1	
		Absent	24 (57%)	20 (49%)	0.71 (0.30-1.70)	0.45	0.96 (0.38-2.44)	0.93

[a]HPV data obtained from previous studies (3, 6, 23). [b]Intensity of staining. [c]Clinical outcome as defined in the Materials and Methods. [d]Effects estimated using logistic regression. [e]Overall test for effect modification (*i.e.* interaction between HPV status and clinical outcome).

classification for Tyr 1068 staining on HPV$^-$ TSCC. However, since this correlation was not significant when the T-classification was dichotomized into T1+T2 and T3+T4, we do not consider this correlation to be valid. Furthermore in some other studies no correlation between EGFR or pEGFR staining and tumor characteristics was found (25, 32). In contrast, some studies have found a significant correlation between EGFR and tumor characteristics, *e.g.* for N classfication and stage for Tyr1068 staining in HNSCC (19). It should be noted that Keller *et al.* found a significant correlation between the presence of the truncated variant EGFR vIII and both T-classification and stage (32).

Knowledge of the presence of EGFR in TSCC may be of importance in the choice of treatment for patients. In the present study, all patients included were diagnosed from 2000-2006 and the vast majority (95%) only received RT. However, due to the poor clinical outcome for patients with HNSCC in general, treatment has since then been intensified and now often includes EGFR inhibitors, such as cetuximab, and treatment outcome may, in part, be influenced by the EGFR status of the tumors. It is important to note that in our study, treatment for patients did not include any EGFR inhibitors. It is possible that a correlation between EGFR and clinical outcome would have been found if the patients had received such therapy.

In two previous studies of partially the same TSCC samples we demonstrated a strong correlation, both for human leukocyte antigen (HLA) A, B and C and tumor-infiltrating CD8$^+$ T-cells, with patient disease-free survival (22, 33). Thus, these markers are potentially more useful in predicting the response of patients to treatment, rather than expression of EGFR or pEGFR, at least for patients receiving RT without adjuvant therapy.

There are some limitations in the present study. It is a retrospective study with a limited sample size. However, in the original cohort all patients that were diagnosed with

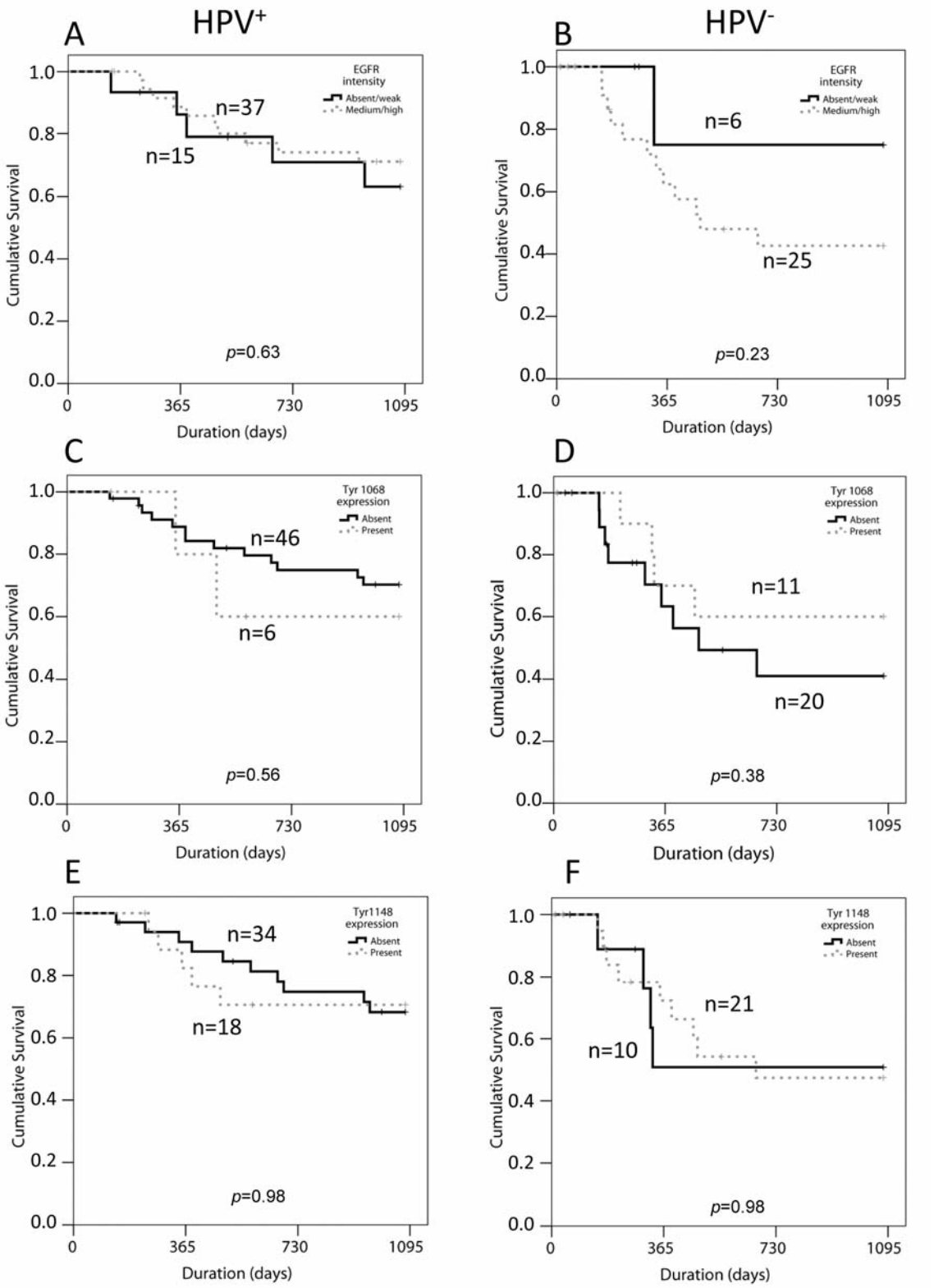

Figure 2. *Disease-free survival analyzed by epidermal growth factor receptor (EGFR) and human papillomvirus (HPV) status of tonsillar squamous cell carcinoma (TSCC), as visualized by the Kaplan–Meier curves. HPV+ (A) and HPV– (B) TSCC stratified by the intensity of EGFR staining; HPV+ (C) and HPV– (D) TSCC stratified by the presence of pEGFR Tyr1068 staining; HPV+ (E) and HPV– (F) TSCC stratified by the presence of pEGFR Tyr1148 staining. n Denotes the number of patients in each stratified group and crosses signify censored patients.*

TSCC in Stockholm during 2000-2006 and treated with intent to cure were included. However, a strength of the present study is that in comparison to most other studies on EGFR in HNSCC, it was performed on relatively homogenous material, where all samples were from the same head and neck subsite.

In summary, our study demonstrates both pEGFR Tyr1068 and Tyr1148 to be correlated with absence of HPV in TSCC, but not to be useful as biomarkers for treatment outcome in patients with TSCC treated by RT without EGFR inhibitors.

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