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Human papillomavirus, smoking status and outcomes in tonsillar squamous cell carcinoma

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It is now clear that the two separate entitles of tonsillar cancer, HPV induced and non-HPV induced (smoking induced), have significantly different presenting stage and outcomes. A significant proportion of patients with human papillomavirus positive tonsillar cancer have had exposure to smoking. We examined the combined effect of human papillomavirus and smoking on the outcomes and determined whether smoking can modify the beneficial effect of human papillomavirus. A total of 403 patients from nine centers were followed up for recurrence or death for a median of 38 months. Determinants of the rate of loco-regional recurrence, death from tonsillar cancer and overall survival were modeled using Cox regression. Smoking status was a significant predictor of overall survival ($p = 0.04$). There were nonstatistically significant trends favoring never smokers for loco-regional recurrence and disease specific survival. In addition, there was no statistically significant interactions between smoking and human papillomavirus (p-values for the interaction were 0.26 for loco-regional recurrence, 0.97 for disease specific survival and 0.73 for overall survival). The effect of smoking on loco-regional recurrence and disease specific survival outcomes was not statistically significant, nor was there significant evidence that the effect of smoking status on these outcomes was modified by HPV status. Irrespective of HPV status, however, smokers did have poorer overall survival than never-smokers, presumably due to effects of smoking that are unrelated to the primary cancer.

Human papillomavirus virus (HPV) is now the main aetiological factor of oropharyngeal squamous cell cancer in Western countries.[1–3] HPV-induced oropharyngeal cancer has distinct clinicopathological features and its prognostic significance is well established.[4] After adjustment for clinicopathological variables, a 58% reduction in death rate has been reported in stage 3 and 4 HPV-positive oropharyngeal cancer.[5] There is evidence that the better outcome associated with HPV is due to a better response to radiation therapy and chemotherapy as well as surgery.[6,7]

In Western countries, there has been a recent increase in incidence for HPV-positive oropharyngeal cancer[2,8] and a decline in smoking prevalence.[9,10] Smoking is known to increase all-cause and cancer-specific mortality in general.[11,12] Specifically, smoking during radiotherapy for head and neck cancer is associated with a lower rate of complete response and poorer overall survival.[13] Chen et al. recently reported lower 5-year loco-regional control, disease-free survival and overall survival among patients with head and neck cancer who continued to smoke after diagnosis compared with smokers who had quit.[14] Patients with HPV-positive oropharyngeal

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What's new?
Smoking is a major risk factor for oropharyngeal cancer, but its impact on prognosis remains unclear. Likewise, there is uncertainty about whether smoking exposure impacts survival specifically for tonsillar cancers caused by human papillomavirus (HPV). While smoking was found to predict overall survival in this follow-up study of more than 400 patients with squamous cell carcinoma of the tonsil, interactions between HPV and smoking were statistically insignificant. Furthermore, the effect of smoking status on locoregional control and disease-specific survival outcome was of limited statistical importance.

cancer are less likely than those with HPV-negative cancer to have smoked.[13,15] However, recent studies have shown that up to one-third of patients with HPV-positive cancer have had exposure to smoking.[15,16] There is conflicting evidence on the effect of smoking on response of HPV-positive cancer to treatment with some studies suggesting that the favorable outcome associated with HPV may be compromised.[15–17] Two phase 2 trials in oropharyngeal cancer suggested that the risk of progression and death increased directly as a function of tobacco exposure at diagnosis and the effect was independent of HPV status.[17] However, two recent studies have shown that smoking alone is not a prognostic factor in the setting of HPV.[18,19] Zhao et al. examined the survival of 135 patients with HPV cancer and showed that smoking was not prognostic factor for progression-free survival and overall survival.[19] This lack of consensus on relationships between smoking, outcomes and HPV status in oropharyngeal cancer reflects factors such as particular clinical endpoint, sample size and statistical analysis. We have, therefore, undertaken a follow-up study of patients with tonsillar cancer to further examine the combined effect of HPV status and smoking on loco-regional control, disease specific survival and overall survival.

Material and Methods

Study population

The initial study group comprised 411 patients with squamous cell carcinoma of the tonsil treated with curative intent at nine Australian hospitals between 1987 and 2006. The study was approved by the ethics committees of all participating hospitals. Data were retrieved by review of institutional databases and patient records. Follow up status were confirmed by treating physicians or the primary physicians. Consecutive patients with accessible tumor material and clinicopathological data were followed up for a median time of 38 months. The study pathologist reviewed the histology and tumor grade in all cases.

Laboratory studies

An HPV-positive cancer was defined as one testing positive for HPV DNA and with p16 overexpression on immunohistochemistry.[20,21] The presence and type of HPV DNA were determined by a E6-based multiplex tandem PCR assay carried out on two to six 4–5 μm sections of tumor using modifications of the method described by Stanley and Szewezuk[22] as previously described.[23] This assay simultaneously detects and identifies 21 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82, 53, 6, 11 and 26). Measured amounts of equine herpesvirus were used to monitor the efficiency of DNA extraction and removal of PCR inhibitors. Paraffin sections were cut using stringent precautions to avoid cross contamination and water blanks were placed after every fifth tube to detect assay contamination.

p16 protein expression was determined by semiquantitative immunohistochemistry using the JC2 p16 antibody (Neomarkers, USA) as previously described.[23] Staining was strong and diffuse and was essentially all or none. Weak focal staining was recorded as negative. The immunohistochemical staining was evaluated by three independent observers including the study pathologist, all of whom were blinded to the clinical data.

Statistical analyses

Comparisons between the clinical and demographic characteristics of HPV-positive and HPV-negative cancers were undertaken using *t*-tests for continuous variables and chi-square tests for categorical variables. Univariate and multivariate time-to-event analyses were undertaken using Cox proportional hazards regression modeling. The method of Lin, Wei and Ying was used to test the constant proportional hazards assumption.[24] The multivariate analyses examined the effect of smoking status adjusted for age, gender, T classification, N classification, treatment, HPV status, an HPV-by-N stage interaction and an HPV-by-T stage interaction. These covariates were chosen for inclusion in the multivariate model on the basis of the results of a previous analysis.[25] Age was fitted as a continuous covariate and the appropriateness of this was evaluated. All other covariates were fitted as categorical variables. The smoking categories were current smoker, ex-smoker and never-smoker. The treatment categories were radiotherapy alone, radiotherapy plus chemotherapy, surgery plus radiotherapy and surgery. Alcohol status was unavailable for 77 patients and was consequently only included as a categorical covariate in the multivariate models in a series of secondary sensitivity analyses. The alcohol categories were drinker, ex-drinker and non-drinker. Times to loco-regional failure, death from tonsillar cancer and death from any cause were calculated from date of diagnosis. For the analysis of time to loco-regional failure (defined as clinical, radiological and/or pathological evidence of recurrence at the primary site or in the regional nodal area), patients were

censored at last follow-up, death or distal recurrence, whichever was first, or excluded if they had incomplete information on recurrence. For the analysis of time to death with tonsillar cancer, patients were censored at last follow-up or death without tonsillar cancer as applicable, or excluded if it was not known whether they died with or without tonsillar cancer. For the analysis of time to death from any cause, patients were censored at last follow-up if they were not known to have died.

Results

HPV status and type distribution

One hundred and eighty-five (45.9%) cancers were defined as HPV-positive. Type 16 accounted for 94.6% of the HPV-positive cases. Other types included 18, 33 and 35. Eight cases with HPV type 16 positive were also positive for a second HPV type. Two hundred eighteen (53%) cancers were HPV-negative (DNA negative and p16 negative). Sixty-three cases (15.6%) were HPV DNA positive and p16 negative. This group of patients was included in the HPV-negative group. The rationale for this grouping has been previously described.[4] Eight patients with HPV DNA negative and p16 positive cancers were excluded from further analyses because it was not known whether these cancers were induced by an HPV type undetectable by the assay or were truly HPV-negative with p16 upregulation through an HPV unrelated pathway. The proportions of HPV-positive cancers were similar across the participating hospitals (data not shown).

Patient characteristics

Patient characteristics are summarized in Table 1. Relative to patients with HPV-negative cancer those with HPV-positive cancer were significantly younger (mean age 55.0 *vs.* 60.4, $p \leq 0.001$) and were more likely to have lower T classification, higher N classification and higher tumor grade cancer (Table 1).

As shown in Table 1, 28.6% of patients with HPV-positive cancer were never smokers, 41.1% were ex-smokers and 30.3% were current smokers at the time of diagnosis. In contrast, only nine patients (4.1%) with HPV-negative cancer were never smokers at diagnosis; 29.8% were ex-smokers and 66.1% were current smokers ($p = 0.001$). There was no significant difference in alcohol intake according to HPV status of the cancers.

Outcome analyses

Loco-regional events. Table 2 shows the event rates at 3 years (*i.e.*, the approximate median follow-up time). Loco-regional recurrence occurred in 100 patients. Among the patients with HPV-positive cancer, the Kaplan-Meier estimates of loco-regional recurrence rates at 3 years were similar among never smokers, ex-smokers and current smokers (8%, 11% and 12% respectively). However, in patients with HPV-negative cancers, the loco-regional recurrence rates among never smokers, ex-smokers and current smokers were 13%, 32% and 43%, respectively.

Distant events. Thirty-three patients developed distant metastasis as the first site of recurrence. At 3 years follow up, 176 patients had died (103 from tonsillar cancer). Among the patients with HPV-positive cancer, the Kaplan-Meier estimates of disease specific death rates at 3 years for never smokers, ex-smokers and current smokers were 7%, 13% and 19%, respectively, when compared with 22%, 29% and 39%, respectively, for patients with HPV-negative cancers. Among the HPV-positive group, current or ex-smokers had poorer estimates of overall survival than never smokers (78%, 83% and 91%, respectively) at 3 years. The 3-year overall survival rates for patients with HPV-negative cancer were numerically worse than for HPV-positive cancers, particularly for current and ex-smokers (45% and 40%, respectively).

Univariate predictors of loco-regional recurrence, disease specific survival and overall survival are presented in Table 3. Smoking was found to be a highly statistically significant univariate predictor of all endpoints. There was no statistical evidence, however, that the smoking effect was modified by HPV status ($p > 0.05$ for interaction term across all three endpoints).

In the multivariate analyses (Table 4), smoking status was a significant predictor of overall survival only ($p = 0.041$) with the hazard for death being estimated at 0.78 (95% CI: 0.56–1.10) for ex-smokers and 0.42 (95% CI: 0.21–0.87) for never smokers, when compared with current smokers. There were nonstatistically significant trends in reduced risk for ex-smokers and never-smokers, relative to current smokers, for disease specific death and loco-regional recurrence. Similar trends in the estimated effect of smoking status on each endpoint were observed in the secondary analysis that included alcohol status as a covariate, but none was statistically significant. Adjusted analyses within the HPV-positive subgroup of the effect of smoking on the hazard of loco-regional recurrence, disease specific survival and overall survival produced similar results (Table 5).

Discussion

The impact of smoking on the outcome of HPV-positive oropharyngeal cancer has recently come under close scrutiny.[5,15] In our study of 403 patients with tonsillar cancer (45.9% HPV-positive), more than two-thirds of patients with HPV-positive cancer and almost all patients with HPV-negative cancers had exposure to smoking. This pattern is consistent with recent reports from other Western countries.[5,15]

We found statistical evidence that smoking was an important prognostic factor for overall survival, but not loco-regional control or disease specific survival after adjusting for confounding variables. Nor was there statistically significant evidence that the effect of smoking was modified by HPV status. The statistical power of this test of interaction will have been affected by the relatively modest number of events observed among non-smokers (see Table 2). Our results are nevertheless similar to a recent publication on the outcomes

Table 1. Demographic and clinical characteristics of the study population according to the HPV status of the cancer

	HPV-positive ($n = 185$)	HPV-negative ($n = 218$)	p-Value
Age at diagnosis	Mean = 55.0 (range: 31–89)	Mean = 60.4 (range: 39–83)	<0.001
Gender			0.327
Female	39 (21.1%)	55 (25.2%)	
Male	146 (78.9%)	163 (74.8%)	
Smoking status			<0.001
Never-smoker	53 (28.6%)	9 (4.1%)	
Ex-smoker	76 (41.1%)	65 (29.8%)	
Current smoker	56 (30.3%)	144 (66.1%)	
Alcohol status (Missing = 77)			0.201
Non-drinker	22 (14.3%)	15 (7.9%)	
Ex-drinker	11 (7.1%)	15 (7.9%)	
Drinker	116 (75.3%)	147 (77.4%)	
T classification			0.002
1	44 (23.8%)	25 (11.5%)	
2	69 (37.3%)	73 (33.5%)	
3	53 (28.6%)	87 (39.9%)	
4	19 (10.3%)	33 (15.1%)	
N classification			<0.001
0	36 (19.5%)	105 (48.2%)	
1	29 (15.7%)	41 (18.8%)	
2	97 (52.4%)	61 (28.0%)	
3	23 (12.4%)	11 (5.0%)	
Grade			<0.001
1	18 (9.7%)	51 (23.4%)	
2	59 (31.9%)	116 (53.2%)	
3	108 (58.4%)	51 (23.4%)	
Treatment			0.001
Radiotherapy + chemotherapy	49 (26.5%)	34 (15.6%)	
Radiotherapy alone	37 (20.0%)	60 (27.5%)	
Surgery + adjuvant Radiotherapy	94 (50.8%)	99 (45.4%)	
Surgery alone	5 (2.7%)	25 (11.5%)	

Table 2. Event rates at 3 years by smoking and HPV status

Event	Smoking status	HPV-positive	HPV-negative	HR (95%CI)[1]
Loco-regional Recurrence	Current smokers	7/56 (13%) [12%]	57/139 (41%) [43%]	4.2 (1.9 to 9.2)
	Ex-smokers	9/75 (12%) [11%]	18/62 (29%) [32%]	2.9 (1.3 to 6.5)
	Never-smokers	8/52 (15%) [8%]	1/9 (11%) [13%]	0.6 (0.1 to 4.6)
Disease specific death	Current smokers	11/56 (20%) [19%]	53/143 (37%) [39%]	2.3 (1.2 to 4.3)
	Ex-smokers	13/75 (17%) [13%]	19/62 (31%) [29%]	2.0 (1.0 to 4.1)
	Never-smokers	5/53 (9%) [7%]	2/9 (22%) [22%]	1.9 (0.4 to 10.2)
Death (any cause)	Current smokers	20/56 (36%) [22%]	86/144 (60%) [45%]	2.1 (1.3 to 3.5)
	Ex-smokers	21/76 (28%) [17%]	39/65 (60%) [40%]	2.6 (1.5 to 4.4)
	Never-smokers	7/53 (13%) [9%]	3/9 (33%) [22%]	1.4 (0.3 to 6.7)

Kaplan-Meier estimate of 3-year event rate shown in square brackets.
[1]Hazard Ratio from proportional hazards regression

Table 3. Univariate analysis

Patient characteristic		Loco-regional recurrence		Disease specific survival		Overall survival	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age at diagnosis	Per 10 year increase in age	1.13 (0.94, 1.36)	0.191	1.21 (1.00, 1.45)	0.049	1.37 (1.18, 1.58)	<0.001
Gender	Female	1.00	0.014	1.00	0.017	1.00	0.024
	Male	2.04 (1.16, 3.58)		1.95 (1.13, 3.37)		1.54 (1.06, 2.25)	
Smoker status	Current smoker	1.00	0.001	1.00	0.002	1.00	<0.001
	Ex-smoker	0.52 (0.33, 0.81)		0.65 (0.42, 0.99)		0.73 (0.53, 1.01)	
	Never smoker	0.34 (0.17, 0.69)		0.28 (0.13, 0.62)		0.25 (0.13, 0.47)	
Alcohol status	Current drinker	1.00	0.038	1.00	0.094	1.00	0.098
	Ex-drinker	0.68 (0.28, 1.68)		1.13 (0.52, 2.45)		1.09 (0.59, 2.03)	
	Never drinker	0.24 (0.07, 0.75))		0.28 (0.09, 0.90)		0.48 (0.24, 0.95)	
HPV status	HPV-negative	1.00	<0.001	1.00	<0.001	1.00	<0.001
	HPV-positive	0.28 (0.18, 0.45)		0.38 (0.25, 0.59)		0.36 (0.26, 0.50)	
T classification	1	1.00	0.027	1.00	<0.001	1.00	<0.001
	2	1.23 (0.63, 2.41)		2.83 (1.10, 7.29)		1.16 (0.68, 1.97)	
	3	1.85 (0.97, 3.53)		4.85 (1.92, 12.22)		2.19 (1.32, 3.62)	
	4	2.49 (1.21, 5.13)		7.71 (2.94, 20.23)		2.92 (1.65, 5.17)	
N classification	0	1.00	0.079	1.00	0.042	1.00	0.345
	1	1.61 (0.97, 2.66)		1.98 (1.13, 3.48)		1.37 (0.90, 2.07)	
	2	0.87 (0.54, 1.40)		1.67 (1.02, 2.72)		1.05 (0.74, 1.49)	
	3	0.69 (0.27, 1.77)		2.29 (1.13, 4.65)		1.42 (0.82, 2.46)	
Grade	1	1.00	0.096	1.00	0.256	1.00	0.098
	2	0.98 (0.59, 1.63)		0.99 (0.60, 1.64)		0.85 (0.58, 1.23)	
	3	0.61 (0.35, 1.07)		0.70 (0.41, 1.21)		0.65 (0.44, 0.97)	

Table 4. Multivariate analysis[1]

Patient characteristic		Loco-regional recurrence		Disease specific survival		Overall survival	
Smoker status	Current smoker	1.00	0.181	1.00	0.104	1.00	0.041
	Ex-smoker	0.64 (0.39, 1.03)		0.69 (0.44, 1.09)		0.78 (0.56, 1.10)	
	Never-smoker	0.83 (0.37, 1.86)		0.45 (0.18, 1.09)		0.42 (0.21, 0.87)	

[1]Adjusted for age, gender, treatment, N stage, T stage, HPV status and HPV-by-stage interactions.

of HPV positive oropharyngeal cancer treated with altered fractionation radiation therapy. In that study, smoking was a predictor for worse overall survival but not cause specific survival.[26] Ang et al. reported the analysis of 323 patients with locally advanced oropharyngeal cancer (63.8% HPV-positive) treated with radiation therapy or chemoradiotherapy. They also showed that HPV status and smoking were strong independent predictors of overall survival and suggested that the outcomes of an HPV-positive cancer may be altered by tobacco use.[5] The highest risk group (HPV-negative cancer, >10 pack-years and any T stage or HPV-negative cancer, <10 pack-years and T4 tumor) was at least five times more likely to die of any cause than the lowest risk group (HPV-positive cancer, <10 pack-years and any N stage or HPV-positive cancer, >10 pack-years and N0-2 stage). However, the effect of smoking on loco-regional control and disease specific survival was not reported in that study. Ang and coworkers also defined an intermediate risk group which included both smokers with higher N stage HPV positive cancer (>10 pack-years, smokers, N2c-3 cancer) and non/light-smokers with lower T stage HPV negative cancer (<10 pack-years, smokers, T2-3 cancer). This risk stratification is contrary to our data suggesting that T stage is most relevant for HPV-positive cancers, and N stage was more relevant for HPV-negative cancers.[25]

A recent report by Gillison et al. on 190 patients with oropharyngeal cancer from two phase 3 trials showed an increase in the risk of progression-free survival and death by

Table 5. Analyses for HPV-positive patients only

	Loco-regional recurrence		Disease specific survival		Overall survival	
Smoking status	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Univariate analysis						
Current smoker	1.00	0.926	1.00	0.288	1.00	0.082
Ex-smoker	0.91 (0.34, 2.45)		0.86 (0.38, 1.91)		0.76 (0.41, 1.42)	
Never-smoker	1.10 (0.40, 3.05)		0.43 (0.15, 1.25)		0.37 (0.16, 0.88)	
Multivariate Analysis[1]						
Current smoker	1.00	0.621	1.00	0.376	1.00	0.295
Ex-smoker	1.39 (0.44, 4.44)		0.87 (0.34, 2.22)		0.87 (0.43, 1.77)	
Never-smoker	1.82 (0.55, 6.02)		0.43 (0.13, 1.45)		0.46 (0.17, 1.23)	

[1]Adjusted for age, gender, treatment, N classification and T classification.

1% per pack-year or 2% per year of smoking independent of HPV status of cancer.[17] Smoking also significantly impacted the risk of locoregional failure. However in that study, never smokers (30.2% of patients with HPV positive cancer) were grouped with smokers with $\leq$10 pack-years in the outcomes analysis despite evidence that patients who continue to smoke during radiation therapy for head and neck cancer have a significantly lower rate of response and survival.[13,14] In contrast, our analysis treated never smokers as a discrete group; however, since our study lacked data on pack-years we were unable to assess the effect of smoking dose on outcomes.

In a recent prospective study of 124 patients with locally advanced oropharyngeal cancer (82.3% HPV-positive) treated with chemoradiotherapy in two cohorts enrolled in two consecutive treatment protocols, Maxwell et al. reported time to recurrence (defined as loco-regional recurrence, distant recurrence as well as second primary cancer) as its primary endpoint.[15] Current smokers with HPV-positive cancer were at significantly higher risk of disease recurrence relative to never users. However, after adjusting for cohort effect, the smoking status of those with HPV-positive cancers did not significantly affect the risk of disease recurrence, disease specific or overall survival. Other studies have reported no interaction between smoking and HPV in relation to survival.[27–30] Inconsistencies in the effect of smoking on outcome in HPV-positive cancers are likely to reflect factors such as differences in primary end point, population characteristics including stage of disease, subsite within the oropharynx and sample size.

The effect of smoking remained statistically significant in our multivariate analyses, with the hazard of death for never-smokers estimated to be less than half that for current smokers. Trends favoring never-smokers were observed for the other endpoints but these did not achieve statistical significance in the multivariate analyses; possibly a result of diminished statistical power as there were fewer events on the loco-regional recurrence and disease specific survival endpoints compared to the overall survival endpoint. The poorer overall survival in smokers is likely due to the detrimental effect of smoking on general health (and perhaps the development of a second primary malignancy). We were not able to examine the effect of smoking cessation for those current smokers who ceased smoking after the diagnosis and lacked data on the development of second primary cancer.

Alcohol consumption is also an aetiological factor for the development of non-HPV related tonsillar cancer.[16] In our cohort, there was no significant difference in alcohol intake at the time of diagnosis according to the HPV status of the cancer. Alcohol intake was not a significant factor for outcomes in univariate analysis. However, our data on alcohol intake were less robust than smoking data due to the difficulty in distinguishing regular heavy drinkers from casual drinkers retrospectively, and therefore, we were not able to examine the relationship between the amount of alcohol intake and outcomes. In addition, socioeconomic status is associated with outcome in head and neck cancer.[31] We do not have the socioeconomic status of our cohort. However, the association between socioeconomic status and outcome in head and neck cancer is a complex issue and is related to smoking as well as delayed presentation, more advanced stage at diagnosis, comorbidity, access to a health care system and less likelihood to receive adjuvant therapy.[32]

In conclusion, the effect of smoking status on loco-regional control and disease specific survival outcomes was not statistically significant in this cohort of patients with squamous cell carcinoma of the tonsil, nor was there significant evidence that the effect of smoking status on these outcomes was modified by HPV status. Irrespective of HPV status, however, smokers did have poorer overall survival than never-smokers, presumably due to effects of smoking that are unrelated to the primary cancer. Cessation of smoking by current smokers at diagnosis may, therefore, be of benefit, as may the optimal management of the detrimental effect of smoking on general health.

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References

1. Chaturvedi AK, Engels EA, Anderson WF, et al. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol* 2008;26:612–9.
2. Hong AM, Grulich AE, Jones D, et al. Squamous cell carcinoma of the oropharynx in Australian males induced by human papillomavirus vaccine targets. *Vaccine* 2010;28:3269–72.
3. Marur S, Forastiere AA. Head and neck cancer: changing epidemiology, diagnosis, and treatment. *Mayo Clin Proc* 2008;83:489–501.
4. Hong AM, Dobbins TA, Lee CS, et al. Human papillomavirus predicts outcome in oropharyngeal cancer in patients treated primarily with surgery or radiation therapy. *Br J Cancer* 2010;103:1510–7.
5. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;363:24–35.
6. Lassen P, Eriksen JG, Hamilton-Dutoit S, et al. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. *J Clin Oncol* 2009;27:1992–8.
7. Rischin D, Young RJ, Fisher R, et al. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol* 2011;28:4142–8.
8. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011;29:4294–301.
9. Pierce JP, Messer K, White MM, et al. Prevalence of heavy smoking in California and the United States, 1965–2007. *JAMA* 2011;305:1106–12.
10. Monteiro CA, Cavalcante TM, Moura EC, et al. Population-based evidence of a strong decline in the prevalence of smokers in Brazil (1989–2003). *Bull World Health Organ* 2007;85:527–34.
11. Doll R, Peto R, Boreham J, et al. Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ* 2004;328:1519.
12. Doll R, Peto R, Boreham J, et al. Mortality from cancer in relation to smoking: 50 years observations on British doctors. *Br J Cancer* 2005;92:426–9.
13. Browman GP, Wong G, Hodson I, et al. Influence of cigarette smoking on the efficacy of radiation therapy in head and neck cancer. *N Engl J Med* 1993;328:159–63.
14. Chen AM, Chen LM, Vaughan A, et al. Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavorable outcome. *Int J Radiat Oncol Biol Phys* 2011;79:414–9.
15. Maxwell JH, Kumar B, Feng FY, et al. Tobacco use in human papillomavirus-positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence. *Clin Cancer Res* 2010;16:1226–35.
16. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst* 2008;100:407–20.
17. Gillison ML, Zhang Q, Jordan R, et al. Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. *J Clin Oncol* 2012;30:2102–11.
18. Laco J, Nekvindova J, Novakova V, et al. Biologic importance and prognostic significance of selected clinicopathological parameters in patients with oral and oropharyngeal squamous cell carcinoma, with emphasis on smoking, protein p16(INK4a) expression, and HPV status. *Neoplasma* 2012;59:398–408.
19. Zhao N, Ang MK, Yin XY, et al. Different cellular p16(INK4a) localisation may signal different survival outcomes in head and neck cancer. *Br J Cancer* 2012;107:482–90.
20. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus—associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol* 2006;24:736–47.
21. Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer* 2007;121:2465–72.
22. Stanley KK, Szewczuk E. Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. *Nucleic Acids Res* 2005;33:e180.
23. Hong AM, Dobbins TA, Lee CS, et al. Use of cyclin D1 in conjunction with human papillomavirus status to predict outcome in oropharyngeal cancer. *Int J Cancer* 2010;128:1532–45.
24. Lin DY, Wei LJ, Ying Z. Checking the Cox model with cumulative sums of martingale-based residuals. *Biometrika* 1993;80:557–72.
25. Hong AM, Martin A, Armstrong BK, et al. Human papillomavirus modifies the prognostic significance of T stage and possibly N stage in tonsillar cancer. *Ann Oncol* 2012. doi: 10.1093/annonc/mds205.
26. O'Sullivan B, Huang SH, Perez-Ordonez B, et al. Outcomes of HPV-related oropharyngeal cancer patients treated by radiotherapy alone using altered fractionation. *Radiother Oncol* 2012;103:49–56.
27. Applebaum KM, Furniss CS, Zeka A, et al. Lack of association of alcohol and tobacco with HPV16-associated head and neck cancer. *J Natl Cancer Inst* 2007;99:1801–10.
28. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;356:1944–56.
29. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008;100:261–9.
30. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000;92:709–20.
31. Robertson G, Greenlaw N, Bray CA, et al. Explaining the effects of socio-economic deprivation on survival in a national prospective cohort study of 1909 patients with head and neck cancers. *Cancer Epidemiol* 2010;34:682–8.
32. Alberto Q, Roberto L, Carlo M, et al. Socio-economic inequalities: a review of methodological issues and the relationships with cancer survival. *Crit Rev Oncol Hematol*, in press; http://dx.doi.org/10.1016/j.critrevonc.2012.08.007.