B-ENT, 2014, **10**, 7-14

HPV-related oropharyngeal cancers in Flanders (Belgium): a multicenter study

E.J. Van Limbergen[1,2], R. Dok[2], A. Laenen[3], E. Hauben[4], D. Van den Weyngaert[5], M. Voordeckers[6], W. De Neve[7], F. Duprez[7], S. Nuyts[1,2,]

[1]Department of Radiation Oncology, UH Leuven, Leuven, Belgium; [2]Lab of Experimental Radiotherapy, KU Leuven, Leuven, Belgium; [3]Interuniversity Institute for Biostatistics and Statistical Bioinformatics, KU Leuven, Leuven, Belgium; [4]Department of Pathology, UH Leuven, Leuven, Belgium; [5]Department of Radiation Oncology, ZNA Middelheim, Antwerp, Antwerp Belgium; [6]Department of Radiation Oncology, UH Brussels, Jette, Brussels, Belgium; [7]Department of Radiation Oncology, UH Ghent, Ghent,Belgium

Key-words. Epidemiology, oropharyngeal neoplasms, Belgium, radiotherapy, human papillomavirus

Abstract. *HPV-related oropharyngeal cancers in Flanders Belgium): A multicenter study. Introduction:* Human papilloma virus (HPV) was recently reported to play a major role in oropharyngeal carcinoma. Large geographical differences in the disease prevalence have been described. Until now, no data have been reported for Flanders (Belgium).
Methods: A multicenter cooperative study was undertaken at the radiation-oncology departments of Flemish universities. Tumor blocks from patients diagnosed with oropharyngeal carcinoma between 2000 and 2010 were tested for HPV at a single center. Patients' characteristics, treatments, and follow-up data were recorded from medical files. Age standardized incidence rates of oropharyngeal carcinoma were collected from the Belgian Cancer Registry.
Results and conclusions: The incidence of oropharyngeal carcinoma has increased in males and females. Tissues were collected from 264 patients and the HPV status could be defined in 249 of them. The prevalence of HPV(+) oropharyngeal carcinoma was 24.78% (19.93-30.36%). In our cohort, HPV(+) tumors occurred in patients with more advanced tumor stages ($p < 0.05$), who smoked less ($p < 0.05$), consumed less alcohol ($p < 0.05$), had a tonsillar/base of tongue sublocalization ($p < 0.05$), and were older ($p < 0.05$). After radiotherapy, locoregional control and disease free survival were significantly better for patients with HPV(+) status ($p < 0.05$) in univariate analysis. HPV status remained a strong predictor of better locoregional control after multivariate analysis. We found that concurrent chemotherapy had an equal benefit for locoregional control in both HPV(+) and HPV(-) patients.

Introduction

Oropharyngeal carcinoma accounts for 23% of all head and neck cancers in Flanders, Belgium with 304 new diagnoses in 2010.[1] Data from the American National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) show a decreasing trend in the overall incidence of head and neck squamous cell carcinomas (HNSCC), which has been attributed to a decrease in the prevalence of smoking.[2] Nonetheless, the incidence of oropharyngeal carcinoma has increased 2-3% annually in the United States.

Tobacco and alcohol use are the primary risk factors for HNSCC and are associated with the majority of these tumors worldwide. Recently, however, high-risk human papillomaviruses (HPV), in particular HPV16, were recognized as independent risk factors strongly associated with oropharyngeal carcinomas.[3] American studies have shown that 40 to 65% of oropharyngeal HNSCC could be attributed to HPV16.[4-7] These HPV infections might be the cause of the increased incidence of oropharyngeal carcinoma. Patients with HPV-associated HNSCC tend to be non-smokers and non-drinkers, present with more advanced N-stage lesions, are diagnosed at a younger age, and ultimately experience improved survival.[3,7] These findings were recently confirmed in correlative studies from the Radiation Therapy and Oncology Group (RTOG)

This work was supported by the clinical research fund (KOF) of the UH Leuven, the Flemish League against Cancer (VLK), and the King Baudouin Foundation (Anhaive Fund) (S.N.). E.V.L. is a research assistant (aspirant) from the Research Foundation Flanders (FWO). None of the funding sources were involved in the process of data collection, interpretation, writing, editing, or reviewing of the presented material. We acknowledge the Belgian Cancer Registry for providing us with their data.

0129 and the Trans Tasman Radiation Oncology group (TROG) 02.02 phase III trials.[8,9] Geographical differences in the disease prevalence seem to exist. In the United States, 40 to 80% of oropharyngeal tumors are associated with HPV; while in Europe, the proportion varies from 90% in Sweden to about 20% in communities with high tobacco exposure.[10] Until now, no data were available for Flanders or Belgium.

Materials and methods

Subjects

The study was a joint effort of the Flemish radiation oncology centers of Antwerp, Brussels, Ghent, and Leuven. The study protocol was approved by the ethical committee of the university hospital of Leuven. All patients registered with oropharyngeal squamous cell carcinoma between 2000 and 2010 were included if formalin fixed paraffin embedded (FFPE) tumor tissue was available. The following data were collected from patients: identification number, date of birth, gender, tobacco and alcohol usage as noted in the medical records, oropharyngeal subsite, TNM and tumor stage (UICC 7th edition), type of therapy, start- and end-date of therapy, date of local or regional relapse, date of distant recurrence, date of death, and date of last follow-up. For survival analysis, only patients who underwent radiotherapy ($\geq$ 60 Gy) for a macroscopic tumor were included.

Tumor samples and laboratory studies

The HPV-status was determined for all patients in one central location (Leuven). HPV testing was performed using FFPE tissue and a previously validated algorithm using data from p16 immunohistochemistry (IHC) and HPV-PCR.[11,12] A tumor was regarded as HPV-related when both p16 IHC and HPV-PCR were positive. From each FFPE block, several sections were cut, using a fresh microtome for each block to avoid DNA carry over. The first and the last sections were H&E stained to confirm the presence of tumor in the biopsies used. A third slice was used for p16 IHC, and another 3 slices were used for DNA extraction and PCR testing. The p16 IHC was performed on 5 µm sections. Deparaffinization, antigen retrieval, and IHC were performed using an automated protocol on a DAKO PT link module and autostainer. For p16 IHC, a purified mouse anti-human p16 antibody (G175-405, BD Pharmigen) was used. Sections were scored as p16 positive when there was clear immunoreactivity in at least 50% of cells.[8,13]

DNA was extracted from FFPE sections using the QIAamp DNA FFPE Tissue kit. The concentration and purity were then determined by spectrophotometry (Nanodrop ND-1000). The quality of the extracted DNA for use as a PCR-template was verified by generating a 167 bp fragment of the IDH2 gene using PCR. Subsequently, HPV status was determined with a PCR reaction using the GP5+/6+ primer set, capable of detecting at least 16 high risk-HPV subtypes in addition to the most common HPV16 and HPV18 subtypes.[14]

Statistics

The confidence interval for HPV prevalence is based on Wilson (score) confidence limits for a binomial proportion. Inverse probability weighting (IPW) was used to deal with non-random, missing HPV statuses. Differences between HPV(-) and HPV(+) groups were analyzed using the Chi-square test in cases of categorical predictors; whereas, the Mann-Whitney U-test was used for ordinal or continuous predictors. Follow-up summary statistics were based on the Kaplan-Meier estimate of potential follow-up. Overall survival (OS) was defined as the time between the start of radiotherapy and death. Disease free survival (DFS) was defined as the time between the start of radiotherapy and disease recurrence or death. Locoregional control (LRC) was defined as the time between the start of radiotherapy and the date of locoregional recurrence. Differences between groups regarding survival or other time-to-event outcomes were analyzed using the Log Rank test. Kaplan-Meier estimates were obtained for survival curves and follow-up. For the analysis of independent predictor effects on time-to-event outcomes, multivariable Cox proportional hazards models were used, including the predictor of interest as well as possible confounders. Changes in incidence rates over time were calculated using the Estimated Annual Percentage Change method. Analyses were performed using SAS software, version 9.2 of the SAS System for Windows. All tests are 2-sided and a 5% significance level was used.

Results

Baseline patient characteristics

In total, 264 patients with an oropharyngeal squamous cell carcinoma were included and their medical records and FFPE tissue were obtained. Baseline patient characteristics are presented in Table 1. The mean follow-up time for our cohort was 4.44 years (2.41 years Q1, 6.63 years Q3). The majority of patients were diagnosed at an advanced disease stage: 51 (19%) and 192 (72%) patients had UICC stage III and IV disease, respectively. The median age of the study population was 58.8 years, ranging from 26 to 83 years. The most frequent tumoral localizations in the oropharyngeal

Table 1

Characteristic	**All patients (*N* = 264)**		**HPV neg. (*N*=196)**		**HPV pos. (*N* = 53)**		**p-value**
HPV status, N (%)							
• Negative	196	(74%)	196		/		
• Positive	53	(20%)	/		53		
• Unknown*	15	(6%)	/		/		
Gender							
• Male	211	(80%)	159	(81%)	41	(77%)	0.73
• Female	53	(20%)	37	(19%)	12	(23%)	
Age, Median (Q1, Q3)	58.8	(52.2, 65.8)	58.0	(51.3,65.2)	62.1	(53.3, 71.1)	0.028
UICC stage number, *N* (%)[$]							
• I	5	(2%)	4	(2%)	1	(2%)	0.026
• II	16	(6%)	14	(7%)	2	(4%)	
• III	51	(19%)	43	(22%)	5	(9%)	
• IV	192	(73%)	135	(69%)	45	(85%)	
Smoking, *N* (%)							
• Never	28	(112%)	11	(6%)	14	(31%)	<0.001
• Stopped >1 year	35	(15%)	22	(13%)	8	(18%)	
• Current	167	(73%)	139	(81%)	23	(51%)	
• Unknown	34		24		8		
Alcohol, current use, *N* (%)							
• No	46	(24%)	25	(18%)	18	(49%)	<0.001
• Weekly	19	(10%)	7	(5%)	9	(24%)	
• Daily	124	(66%)	108	(77%)	10	(27%)	
• Unknown	75		56		16		
Localization, *N* (%)							
• Soft palate	12	(5%)	11	(6%)	0	(0%)	0.002
• Tonsil	114	(46%)	72	(40%)	33	(66%)	
• BOT/vallecula**	88	(36%)	67	(37%)	16	(32%)	
• Pharyngeal wall	31	(13%)	30	(17%)	1	(2%)	
• Unclear	19		16		3		
Concurrent treatment, *N* (%)							
• None	100	(39%)	78	(40%)	16	(32%)	0.53
• Chemo	152	(58%)	111	(57%)	32	(64%)	
• EGFR inhibition	7	(3%)	5	(3%)	2	(4%)	
• Censored (no radiotherapy or missing information)	5		2		3		
OTT[#], Median (Q1, Q3)	44.0	(42.0, 47.0)	44.0	(42.0,47.0)	44.0	(42.0,48.0)	0.45

Differences between HPV- and HPV+ groups were analyzed using the Chi square test for gender, tonsillar localization, and concurrent treatment. The Mann-Whitney U test was used for age, UICC stage number, pack-years, alcohol use, OTT. * For some patients HPV status could not be determined. The characteristics of this group are not shown; therefore, the sum of the HPV positive and negative subgroups is equal or lower than the number shown for the whole patient population. ** BOT, base of tongue. [$]UICC 7th edition. [#]OTT, Overall Treatment Time.

subsite were tonsillar and base of tongue (BOT), for 114 (43%) and 88 (33%) patients, respectively. Of 264 patients, 213 (75%) received ablative doses of radiotherapy (≥ 60 Gy) for a macroscopic tumor. The remaining 51 patients received postoperative radiotherapy (35), no radiotherapy (4), palliative radiotherapy (2), or stopped radiotherapy prematurely (10). In addition, 152 (58%) patients were treated with concurrent chemotherapy and seven patients (3%) received concurrent treatment based on EGFR inhibition. Adequate information regarding smoking and ethanol use was retrieved from the medical files for 230 (87%) and 189 (72%) patients, respectively.

Incidence trends of head and neck carcinoma and oropharyngeal carcinoma in Flanders: from 1999 to 2010

From the Belgian Cancer Registry (www.kanker-register.org), the age-standardized incidence rates (WSR) for males and females between 1999 and 2010 were obtained for head and neck cancer in general, and more specifically for the oropharyngeal subsite, to investigate incidence trends over time in Flanders. As shown in Figure 1, the incidence of head and neck cancer decreased in males (-1.1% per year) and increased in females (+2.8% per year); whereas, the incidence rate (N/100.000) for oropharyngeal cancers increased significantly in both males (+2.3% per year) and females (+5.5% per year).

Prevalence of HPV-related oropharyngeal disease

For a small number (15 cases, see Table 1) of observations, there was missing information on HPV status. In 2 of 15 cases there was no information on p16 IHC. In the other 13 cases, there was positive p16 IHC, but no HPV PCR results (e.g., due to an insufficient amount or insufficient quality of extracted DNA). Patients with negative p16 IHC were regarded as HPV(-).[11] In case of random missingness (equal probability of unknown HPV status for HPV(-) and HPV(+)), missing information did not affect the estimate of the prevalence. However, as a result of the applied algorithm, we observed that all cases with unknown HPV status, but known p16 status, had a positive p16 status. This suggested that the probability of unknown HPV status is larger for HPV(+) compared to HPV(-) patients; therefore, the prevalence of HPV may be underestimated if we only use the observed data. To account for this, we estimated prevalence by accounting for missing data using the means of IPW. Based on this analysis, the

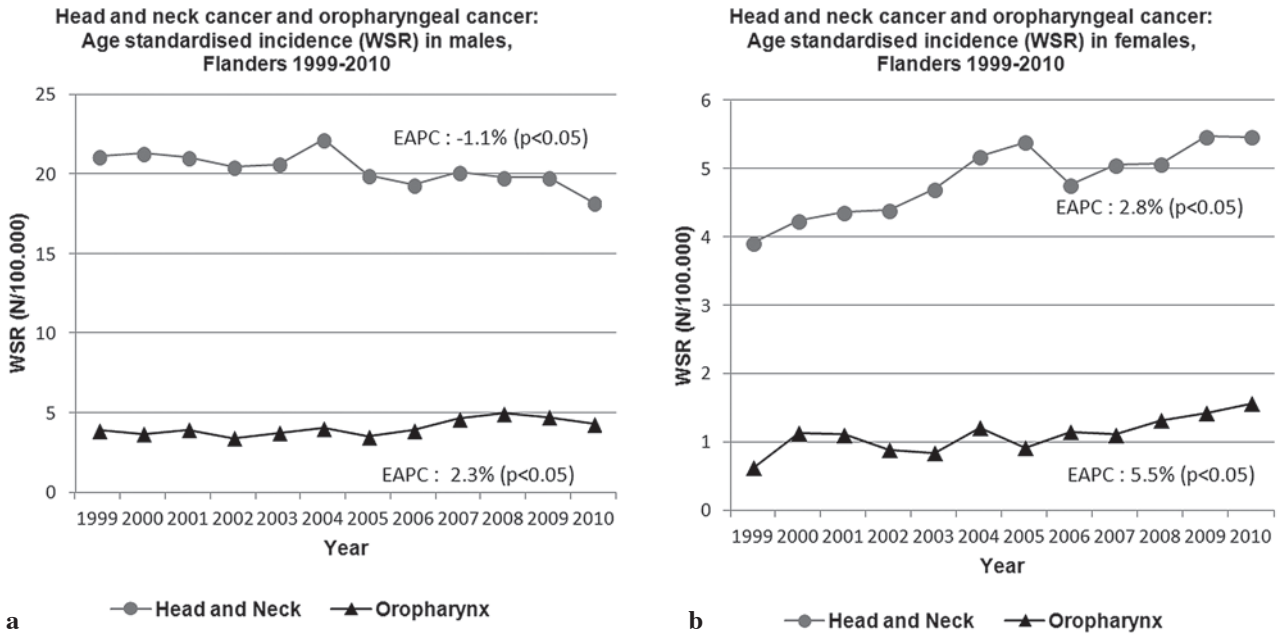

Figure 1

Incidence rates of head and neck cancers in total and cancers of the oropharyngeal subsite according to gender. Data are based on crude incidence rates (N/100.000) for the Flemish region, obtained from the National Cancer Registry (Belgium). EAPC, Estimated Annual Percentage of Change. A p-value < 0.05 indicates a significant change over time.

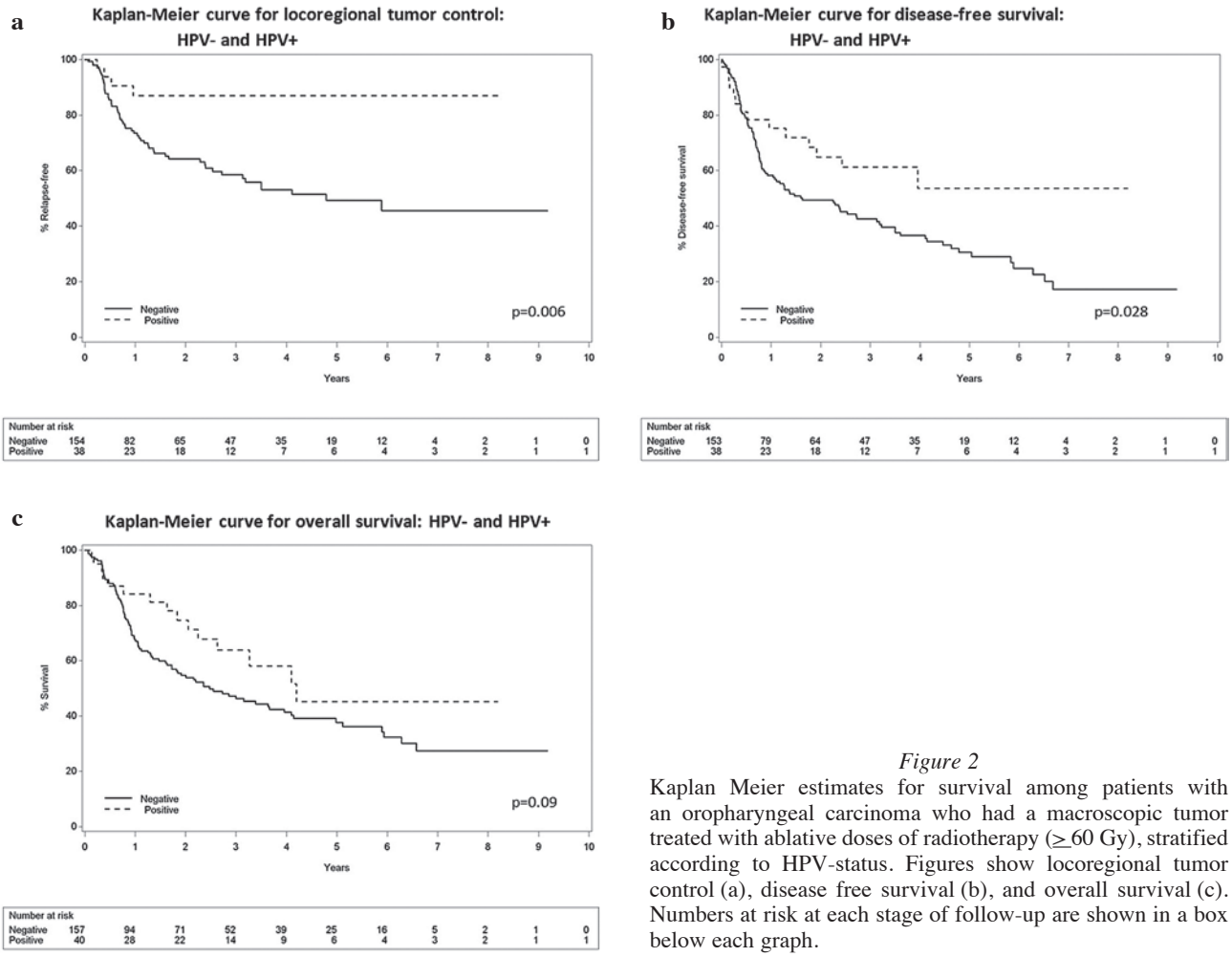

Figure 2
Kaplan Meier estimates for survival among patients with an oropharyngeal carcinoma who had a macroscopic tumor treated with ablative doses of radiotherapy (≥60 Gy), stratified according to HPV-status. Figures show locoregional tumor control (a), disease free survival (b), and overall survival (c). Numbers at risk at each stage of follow-up are shown in a box below each graph.

estimated prevalence of HPV-related squamous cell carcinomas located in the oropharyngeal subsite is 24.78% (19.93-30.36%). Additionally, we observed that HPV(+) cases are significantly ($p < 0.05$) associated with higher UICC tumor stage, lower alcohol and tobacco usage, and a tonsillar or BOT sublocalization in the oropharynx (Table 1). Of note, in our study population, the HPV(+) patients had a significantly older age at diagnosis (62 vs. 58 years, $p = 0.028$). The prevalence of HPV(+) disease was similar in males and females.

Prognostic effect of HPV status after high dose radiotherapy

In this analysis, only patients who received ablative radiotherapy (≥ 60 Gy) for a macroscopic tumor were included. Positive HPV status was associated with a better LRC after radiotherapy at 1 and 5 years (87% and 87%, respectively) when compared to the HPV(-) group (73% and 49%, respectively, $p = 0.006$; Figure 2a). At 1 and 5 years, the DFS was also better in the HPV(+) group (75% and 54%, respectively) in comparison to the HPV(-) patient group (58% and 31%, respectively) ($p = 0.028$; Figure 2b). For OS, we noted a trend for better outcome in the HPV positive group where the OS was 84% at 1 year and 45% at 5 years in comparison to 67% and 38%, respectively, in the HPV negative group ($p = 0.09$; Figure 2c). Finally we estimated the effect of HPV status on outcome, correcting for possible confounders significantly associated with HPV status in a Cox proportional hazards model. Included confounders were tumor stage, age, smoking, drinking, and tumor localization (see Table 1). Since there was no association between concurrent chemotherapy or overall treatment time and HPV-status, they were not included as possible confounders. After correction for the described confounders, positive HPV status remained significantly associated with

better LRC (Hazard Rate (HR) 0.21, p = 0.018); while, the associations between HPV status and DFS/OS were not significant.

The prognostic effect of smoking and concurrent chemotherapy in HPV(+) oropharyngeal carcinoma

Smoking and concurrent chemotherapy are well known parameters affecting the outcome in classical HPV(-) head and neck cancers. It is not clear whether these parameters are equally important in HPV(+) head and neck cancers. To investigate the effect of smoking as an independent predictor of outcome next to HPV status, a multivariate model was built including both smoking and HPV status as predictors. In our series, we found a significantly better DFS for never smokers compared to smokers (HR 0.39, p = 0.036), but not to former smokers. Regarding OS, we also noted a clear trend towards better outcome for never smokers compared to current smokers (HR 0.45, p = 0.072). Smoking did not seem to be an independent risk factor for predicting LRC.

To determine if concurrent chemotherapy was associated with better LRC and OS in HPV(+) patients, the interaction between HPV status and concurrent chemotherapy was tested and found not to exist. Therefore, we also tested for the main effect of concurrent chemotherapy, which was assumed to be equal in HPV(-) and HPV (+) patients. A significantly better LRC was found for concurrent radio-chemotherapy (HR = 0.59, p = 0.0462). We did not find a significant association with OS.

Discussion

While low risk HPV infections may induce benign wart-like lesions predominantly localized on the larynx, high risk HPV infections may induce cancerous lesions predominantly localized in the oropharynx.[3,15] These HPV-associated oropharyngeal carcinomas currently represent an emerging global pandemic. However, large geographical differences in the prevalence seem to exist.[6,10] No data were available for the region of Flanders.

Our analysis of data from the National Cancer Registry of Belgium determined a yearly increase of 2.3% in the incidence rates of oropharyngeal carcinoma for males (Figure 1A) in the region of Flanders, which was comparable to the SEER-data. Yet, the general incidence of head and neck cancers remained stable, which suggests that the higher incidence of oropharyngeal carcinoma was not related to increased exposure of the population to tobacco and alcohol. However, it was possibly attributed to an increase in HPV-infections.

Our series was a multicenter collaboration between the different radiation-oncology departments of the Flemish universities. We documented a prevalence of HPV-induced oropharyngeal carcinoma of 24.78% (19.93-30.36%) by testing with p16 IHC as well as HPV-PCR.[11] These data are comparable to the prevalences reported in our neighboring regions, such as the Netherlands (23.3-29%), France (17.2%), and Germany (16.7-62.9%);[6,12] although, some of these series were conducted earlier and may not represent the current prevalence rates. The rates of HPV-related disease in male and female subgroups were similar.

HPV-related carcinomas were associated with higher tumor stage, lower alcohol or tobacco exposure, and a tonsillar or BOT sublocalization. In addition, survival analysis showed significant improvements for LRC, DFS, and OS in the HPV-related pathology compared with the HPV(-) disease (87% vs. 49%, 54% vs. 31%, and 45% vs. 38%, respectively) at 5 years (Figure 2a-c). After multivariate analysis, HPV remained a strong independent factor associated with better LRC after radiotherapy (HR = 0.21, p = 0.02). These findings are all in agreement with other published series.[3,4,7-9,16,17] On the other hand, we found HPV(+) status in Flemish patients to be associated with older rather than younger age, which is in contrast to many other series. The reason for this remains unclear. Although the incidence age of HPV(-) oropharyngeal cancers in our series seems comparable to those seen in the American series, [4,8] the incidence age of HPV(+) cancers seems older. These findings may be explained by differences in the age exposed to HPV through oral sexual contact.[4,8]

Recent studies suggest that smoking status is a prognostic factor for patients with oropharyngeal carcinoma treated with radiotherapy, and is independent from HPV status.[8,18] We confirmed these observations by showing the DFS was significantly worse for current smokers compared to never-smokers (HR 2.55, p = 0.036). In addition, there was a clear trend towards a worse OS for current smokers compared to never-smokers (HR 2.23, p = 0.072). Concurrent radio-chemotherapy

is a highly toxic treatment; although, it improved the outcome in locoregionally advanced head and neck carcinomas.[19,20] However, a more recent report claimed that radiotherapy alone had similar LRC rates compared to concurrent radio-chemotherapy in advanced stage HPV-associated oropharyngeal carcinoma.[21] Thus, treatment strategies may need to be reevaluated. However, in our series, we did not find any evidence that the effectiveness of concurrent radio-chemotherapy was different for HPV(+) vs. HPV(-) patients. In addition, we noted a significant association towards improvement of LRC with the addition of chemotherapy (HR = 0.59, p = 0.046) for both subgroups.

Conclusion

The prevalence of HPV(+) oropharyngeal carcinoma was 24.78% (19.93-30.36%). HPV(+) tumors correlated with a more advanced tumor stage, less smoking and alcohol consumption, and a tonsillar/BOT sublocalization, but were associated with older age in our cohort. After radiotherapy, LRC, DFS, and OS were significantly better for HPV(+) patients in univariate analysis. HPV status remained a strong predictor of better LRC after multivariate analysis. We also found the addition of concurrent chemotherapy had an equal beneficial effect on LRC in HPV(+) and HPV(-) patients.

Acknowledgements

This work was supported by the clinical research fund (KOF) of the UH Leuven, the Flemish League against Cancer (VLK), and the King Baudouin Foundation (Anhaive Fund) (S.N.). E.V.L. is a research assistant (aspirant) from the Research Foundation Flanders (FWO). None of the funding sources were involved in the process of data collection, interpretation, writing, editing, or reviewing of the presented material. We acknowledge the Belgian Cancer Registry for providing us with their data.

References

1. Belgian Cancer Registry. Available at: http://www.kanker-register.org. Accessed November 22, 2013.
2. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer.* 2007;110(7):1429-1435.
3. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst.* 2000;92(9):709-720.
4. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. 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(4):261-269.
5. Gillison ML, Lowy DR. A causal role for human papillomavirus in head and neck cancer. *Lancet.* 2004;363(9420):1488-1489.
6. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev.* 2005;14(2):467-475.
7. Kumar B, Cordell KG, Lee JS, Worden FP, Prince ME, Tran HH, Wolf GT, Urba SG, Chepeha DB, Teknos TN, Eisbruch A, Tsien CI, Taylor JM, D'Silva NJ, Yang K, Kurnit DM, Bauer JA, Bradford CR, Carey TE. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *J Clin Oncol.* 2008;26(19):3128-3137.
8. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.* 2010;363(1):24-35.
9. Rischin D, Young RJ, Fisher R, Fox SB, Le QT, Peters LJ, Solomon B, Choi J, O'Sullivan B, Kenny LM, McArthur GA. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol.* 2010;28(27):4142-4148.
10. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol.* 2010;11(8):781-789.
11. Smeets SJ, Hesselink AT, Speel EJ, Haesevoets A, Snijders PJ, Pawlita M, Meijer CJ, Braakhuis BJ, Leemans CR, Brakenhoff RH. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer.* 2007;121(11):2465-2472.
12. Rietbergen MM, Leemans CR, Bloemena E, Heideman DA, Braakhuis BJ, Hesselink AT, Witte BI, Baatenburg de Jong RJ, Meijer CJ, Snijders PJ, Brakenhoff RH. Increasing prevalence rates of HPV attributable oropharyngeal squamous cell carcinomas in the Netherlands as assessed by a validated test algorithm. *Int J Cancer.* 2013;132(7):1565-1571.
13. Hafkamp HC, Speel EJ, Haesevoets A, Bot FJ, Dinjens WN, Ramaekers FC, Hopman AH, Manni JJ. A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and overexpression of p16INK4A and p53 in the absence of mutations in p53 exons 5-8. *Int J Cancer.* 2003;107(3):394-400.
14. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol.* 1995;76(4):1057-1062.

15. Ablanedo-Terrazas Y, Soda-Merhy A, Hernández-Palestina M, Ormsby CE, Reyes-Terán G. Intralesional cidofovir in severe juvenile respiratory papillomatosis. *B-ENT.* 2012;8(3):197-202
16. Fouret P, Monceaux G, Temam S, Lacourreye L, St Guily JL. Human papillomavirus in head and neck squamous cell carcinomas in nonsmokers. *Arch Otolaryngol Head Neck Surg.* 1997;123(5):513-516.
17. Lassen P. The role of Human papillomavirus in head and neck cancer and the impact on radiotherapy outcome. *Radiother Oncol.* 2010;95(3):371-380.
18. Hafkamp HC, Manni JJ, Haesevoets A, Voogd AC, Schepers M, Bot FJ, Hopman AH, Ramaekers FC, Speel EJ. Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. *Int J Cancer.* 2008;122(12):2656-2664.
19. Machtay M, Moughan J, Trotti A, Garden AS, Weber RS, Cooper JS, Forastiere A, Ang KK. Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. *J Clin Oncol.* 2008;26(21):3582-3589.
20. Pignon JP, le MA, Maillard E, Bourhis J. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. *Radiother Oncol.* 2009;92(1):4-14.
21. O'Sullivan B, Huang SH, Perez-Ordonez B, Massey C, Siu LL, Weinreb I, Hope A, Kim J, Bayley AJ, Cummings B, Ringash J, Dawson LA, Cho BC, Chen E, Irish J, Gilbert RW, Hui A, Liu FF, Zhao H, Waldron JN, Xu W. Outcomes of HPV-related oropharyngeal cancer patients treated by radiotherapy alone using altered fractionation. *Radiother Oncol.* 2012;103(1):49-56.

Sandra Nuyts, MD, PhD
Department of Radiation Oncology,
UH Leuven
Herestraat 49, 3000 Leuven, Belgium
Tel: +32 16 34 76 00
Fax: +32 16 34 76 23
Email: Sandra.nuyts@uzleuven.be