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Impact of HPV-associated p16-expression on radiotherapy outcome in advanced oropharynx and non-oropharynx cancer ☆

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ABSTRACT

Background and purpose: HPV is found in head and neck cancer from all sites with a higher prevalence in oropharynx cancer (OPC) compared to non-OPC. HPV/p16-status has a significant impact on radiotherapy (RT) outcome in advanced OPC, but less is known about the influence in non-OPC. We analyzed HPV-associated p16-expression in a cohort of patients with stage III–IV pharynx and larynx cancer treated with primary, curatively intended (chemo-)RT, aiming to test the hypothesis that the impact of HPV/p16 also extends to tumors of non-oropharyngeal origin.

Material and methods: 1294 patients enrolled in previously conducted DAHANCA-trials between 1992 and 2012 were identified. Tumors were evaluated by p16-immunohistochemistry and classified as positive in case of staining in >70% of tumors cells.

Results: Thirty-eight percent (490/1294) of the tumors were p16-positive with a significantly higher frequency in OPC (425/815) than in non-OPC (65/479), $p < .0001$. In OPC p16-positivity significantly improved loco-regional control (LRC) (adjusted HR [95% CI]: 0.43 [0.32–0.57]), event-free survival (EFS) (HR 0.44 [0.35–0.56]), and overall survival (OS) (HR: 0.38 [0.29–0.49]), respectively, compared with p16-negativity. In non-OPC no prognostic impact of p16-status was found for either endpoint: LRC (HR: 1.13 [0.75–1.70]), EFS (HR: 1.06 [0.76–1.47]), and OS (HR: 0.82 [0.59–1.16]).

Conclusions: The independent influence of HPV-associated p16-expression in advanced OPC treated with primary RT was confirmed. However, RT-outcome in the group of non-OPC did not differ by tumor p16-status, indicating that the prognostic impact may be restricted to OPC only.

Squamous cell carcinomas of the head and neck (HNSCCs) have traditionally been grouped as a cancer entity, mainly based on the anatomy, common etiology and sensitivity to treatment. HNSCCs arise from the epithelium of the upper aerodigestive tract and as such represent a rather heterogeneous group of neoplasias including carcinomas of the oral cavity, pharynx (oropharynx, nasopharynx and hypopharynx) and larynx. With the emergence of human papillomavirus (HPV) as contributing etiological factor in carcinogenesis of a subgroup of HNSCC [1], the apparent epidemic incidence increase of HPV-associated HNSCC [2–4] and the potent impact on treatment sensitivity and prognosis for patients with HPV-associated disease, the traditional understanding and therapeutic management of HNSCC has been more or less revolutionized. The strongest association with HPV is found in oropharynx cancer (OPC) where tumours of the tonsils are particularly associated with HPV infection [5,6] but high-risk HPV, predominantly HPV-16, has been found in HNSCC from all sites although with a significantly higher prevalence in OPC compared to tumours arising outside the oropharyngeal region (non-OPC) [7,8].

The Danish Head and Neck Cancer Group (DAHANCA) has a longstanding tradition for conducting nationwide randomized trials [9,10] encompassing all eligible Danish HNSCC patients aiming to improve RT-outcome for these patients in general. The use of HPV-associated p16-expression as stratification parameter within these trials has demonstrated that tumor p16-status is presently the strongest known independent factor in primary RT of head and neck cancer [11–17]. Numerous other studies have focused

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on sub-group analysis of patients with OPC within previously conducted randomized trials, concordantly demonstrating a highly significant impact of tumor HPV/p16-status on treatment outcome [18,19]. These observations are believed to be caused in part by a higher sensitivity of HPV/p16-positive tumors to RT [20,21] combined with a different and more favorable risk factor profile [22] and better general health status in the group of patients with HPV/p16-positive disease. Much less is known about the impact of HPV/p16-status in non-OPC although a recent paper indicated, that tumor p16-status also has prognostic value in tumors of non-oropharyngeal origin [23].

Clinical trials are presently investigating whether de-intensified treatment strategies could result in avoidance of excessive toxicity without compromising outcome for selected patients with HPV/p16-positive OPC. On the other hand, the European Organisation for Research and Treatment of Cancer (EORTC) in collaboration with the DAHANCA has launched a trial (EORTC1219 ROG-HNCG/DAHANCA29) investigating whether additional intensification of treatment could be beneficial for patients with HPV/p16-negative HNSCC based on the observed poor outcome for this patient group [24]. Thus presently there is substantial variation in the treatment strategies considered for patients with head and neck cancer dependent of the HPV/p16-status of the tumors. Consequently, provision of further knowledge regarding outcome for patients with p16-positive non-OPC is needed in order to secure optimal and safe treatment for these patients also. Accordingly, in the present study we analyzed the influence of HPV-associated p16-expression in a large cohort of patients with advanced pharynx and larynx carcinoma treated with primary, curatively intended RT, aiming to test the hypothesis that the prognostic impact of HPV/p16 also extends to tumors of non-oropharyngeal origin.

Materials and methods

Study population

The study cohort was identified in the nationwide DAHANCA database which contains information on all Danish head and neck cancer patients since 1970. Since a very high proportion of patients with HPV-associated OPC present with advanced disease stage due to regional lymphnode involvement at time of diagnosis, we selected patients with advanced (stage III–IV) pharynx (oropharynx and hypopharynx) and larynx carcinoma to avoid skewness caused by stage differences between the two groups (OPC vs. non-OPC). All patients were treated with curatively intended primary (chemo)radiotherapy within DAHANCA trials from 1992 to 2012 and the impact of tumor p16-status within these individual trials have been reported previously [11–17]. Due to the specific epidemiology (Epstein Barr virus), histology, growth-pattern and prognosis, nasopharyngeal carcinomas were not included. Moreover, patients with carcinoma of the oral cavity were deliberately excluded since standard treatment in Denmark of these tumors often consists of primary surgery followed by postoperative radiotherapy if indicated. The DAHANCA trials were performed in accordance with the Helsinki Declaration II, and the patients gave written informed consent. Both the main studies and the tumor tissue analyses performed in the present study were approved by the relevant regional and national ethics committees according to Danish legislation and regulations.

Evaluation of p16 immunohistochemistry

Routine paraffin-embedded, formalin-fixed pretreatment tumor tissues were available from 1294 patients and immunohistochemistry (IHC) for p16-expression was performed on whole tumor sections as previously described [11]. Briefly, paraffin sections were cut at 5 μm on Superfrost® plus charged glass slides (Menzel–Glaser), heated at 60 °C for 1 h and deparaffinized in the instrument with EZ prep solution (Ventana Medical Systems). Heat induced antigen retrieval was carried out using Cell Conditioning 1 solution (CC1, Ventana Medical Systems). p16 was detected by incubating sections with antibody clone JC8 (sc-56330; Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted 1:25 for 32 min. JC8 is a mouse monoclonal immunoglobulin G2a antibody raised against full-length human p16, particularly suitable for use on formalin-fixed, paraffin-embedded sections. The specificity was confirmed by Western blotting [11]. Specific reactions were detected using *ultra*View Universal DAB Detection Kit (Ventana Medical Systems), and the slides were counterstained with hematoxylin. Sections of p16-positive cervical carcinoma were used as positive controls. The scoring and classification of the tumors were based in part on a 70% cutpoint but also in consideration of the typical microscopic appearance of an HPV-related tumor in order to optimise the correlation with HPV as described and recommended by El-Naggar et al. for oropharyngeal carcinomas [25]. Up until 2006 the scoring was done retrospectively and a tumor was classified as p16-positive in case of strong, diffuse nuclear and cytoplasmatic staining in more than 70% of carcinoma cells, but tumors displaying a conventional keratinizing morphology together with a variable or patchy p16-expression staining pattern were classified as p16-negative [26]. Since then p16-IHC has been implemented in the routine work-up of patients with HNSCC in Denmark and as such the staining and interpretation of p16-IHC has been performed prospectively by pathologists at the respective oncological centers.

Statistical analysis

The primary endpoint was loco-regional control (LRC) 5 years after completion of RT and secondary endpoints were event-free survival (EFS) [27] and overall survival (OS). LRC was defined as complete and persistent absence of the disease in T-site and regional lymph nodes (N-site) after radiotherapy. Failure was recorded in the event of recurrent tumor, or if the primary tumor never completely disappeared. In the latter situation the tumor was then assumed to have failed at the time of randomization. Consequently the endpoint does not include the effect of a successful procedure with salvage surgery. EFS was defined as loco-regional recurrence or progression, distant metastasis or death resulting from any cause and OS was defined as death resulting from any cause. Regarding LRC patients were followed for at least 5 years and the survival status of the patients was tracked until the end of 2012.

The χ^2-test for categorical variables was used to compare characteristics of p16-positive and negative tumors and OPC vs. non-OPC, respectively. The actuarial values of the endpoints were estimated by the Kaplan–Meier method and compared with a log-rank test. Multivariable hazard ratios (HRs) were estimated by use of the Cox proportional-hazard method to evaluate prognostic parameters and treatment with respect to the risk of loco-regional failure, event-free death and overall death. Additional multivariable analyses were done including an assessment of the interaction between p16-status and tumor site (OPC vs. non-OPC). All results were considered significant at levels less than 5% (two-sided tests) and HRs presented with 95% confidence intervals.

Results

Table 1 shows patient and tumor characteristics according to tumor site and p16-status. Sixty-three percent (815) of the tumors were OPC, and 37% (479) were of non-OPC origin (larynx: 25% (321) and hypopharynx: 12% (158), respectively). Overall, 38%

Table 1
Patient and tumor characteristics by p16-status and tumor-site.

	All patients (n = 1294)	Oropharynx (n = 815)			Non-oropharynx (n = 479)		
		p16-positive (n = 425)	p16-negative (n = 390)	p[a]	p16-positive (n = 65)	p16-negative (n = 414)	p[a]
	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)	
Tumor site							
Oropharynx	815 (63)	425 (100)	390 (100)	<.0001[b]			
Larynx	321 (25)				44 (68)	277 (67)	<.0001[b]
Hypopharynx	158 (12)				21 (32)	137 (33)	
Age (years)							
Median (range)	59 (28–84)	57 (34–83)	58 (28–84)	n.s	59 (34–82)	60 (35–83)	n.s
Gender							
Female	270 (21)	88 (21)	91 (23)	n.s	19 (29)	72 (17)	.02
Male	1024 (79)	337 (79)	299 (77)		46 (71)	342 (83)	
Tumor stage*							
T1–2	619 (48)	279 (66)	166 (43)	<.0001	27 (42)	147 (36)	n.s
T3–4	675 (52)	146 (34)	224 (57)		38 (58)	267 (64)	
Nodal status*							
N0	235 (18)	19 (4)	60 (15)	<.0001	15 (23)	141 (34)	n.s
N+	1059 (82)	406 (96)	330 (85)		50 (77)	273 (66)	
Disease stage*							
III	434 (34)	101 (24)	132 (34)	<.0001	29 (45)	172 (42)	n.s
IV	860 (66)	324 (76)	258 (66)		36 (55)	242 (58)	
Treatment							
5fx/week	380 (29)	71 (17)	118 (30)	<.0001	16 (24)	175 (42)	.007
6fx/week	914 (71)	354 (83)	272 (70)		49 (76)	239 (58)	
No Nimorazole	140 (11)	20 (5)	45 (12)	<.0001	4 (6)	71 (17)	.00
Nimorazole	1154 (89)	405 (95	345 (88)		61 (94)	343 (83)	
No chemotherapy	931 (72)	190 (45)	334 (86)	<.0001	56 (86)	351 (85)	n.s
Chemotherapy	363 (28)	235 (55)	56 (14)		9 (14)	63 (15)	

Abbreviation: n.s, not significant.
* International Union of Cancer Research (UICC) 1982 classification.
[a] Chi-square test for comparison between p16-positive and p16-negative tumors.
[b] Oropharynx vs. non-oropharynx.

(490/1294) of the tumors were p16-positive and the frequency of p16-positivity was significantly higher in OPC (425/815, 52%) than in non-OPC (65/479, 14%), $p < .0001$. The site-specific distribution of p16-positivity in non-OPC was as follows: larynx 44/321 (14%) and hypopharynx 21/158 (13%).

Median age did not differ between groups but in non-OPC there were significantly more women in the p16-positive group compared to p16-negative non-OPC whereas gender was equally distributed in OPC. p16-positive OPC were characterized by small T-size (T1–2) and lymphnode involvement (N+) as opposed to patients with p16-negative OPC whereas such differences were not present in non-OPC. The proportion of OPC increased from 52% in the beginning of the recruitment period whereas late in the period these tumors accounted for approximately 80% of the patients. The corresponding frequency of p16-positivity increased from 22% to 70% in the same period. These findings reflect the demographic shift observed in HNSCC in some parts of the western world, including Denmark [2] and which in all probability can be explained by an epidemic increase in the incidence of HPV-associated p16-positive OPC during the recruitment period. Based on a combination of this demographic shift and the successive implementation of new treatment strategies for HNSCC throughout the recruitment time, significant differences in terms of treatment components were observed in the study cohort, Table 1. Three-hundred and eighty patients were treated with conventionally fractionated radiotherapy (5fx/week) with (N = 240) or without (N = 140) Nimorazole, reflecting the use of this treatment strategy in the beginning of the period. The majority (N = 914) of the patients were treated with moderately accelerated radiotherapy (68 GY/34fx, 6fx/week) concomitant with the hypoxic cell radiosensitizer Nimorazole and a minor proportion of the patients (N = 363) received chemotherapy (weekly Cisplatinum 40 mg/m^2) in addition to moderate acceleration and Nimorazole. This resulted in significant treatment differences between p16-positive and p16-negative tumors of both oropharyngeal and non-oropharyngeal origin, as can be seen in Table 1.

At the time of evaluation, 460 patients had failed to achieve persistent loco-regional control within the irradiated volume. Distant metastasis occurred in 128 patients, 37 in the p16-positive group of which 24 were isolated distant failures and 91 in the p16-negative group of which 39 were distant metastasis alone. A total of 426 patients had died with or from the cancer in question, and overall, 666 patients had died.

In the total cohort of 1294 patients, the 5-year actuarial probability of LRC, EFS and OS was significantly better for patients with p16-positive tumors compared with p16-negativity (LRC: 76% vs. 50%, adjusted HR [95%CI]: 0.51 [0.41–0.65], EFS: 61% vs. 39%, adjusted HR: 0.50 [0.42–0.60] and OS: 74% vs. 36%, adjusted HR: 0.42 [0.34–0.51], respectively). Similarly, in the group of OPC, p16-positivity was correlated with a significant improvement in LRC (80% vs. 52%, HR: 0.32 [0.25–0.43], Fig. 1A) EFS (67% vs. 31%, HR 0.34 [0.27–0.42], Fig. 1C), and OS (80% vs. 37%, HR: 0.26 [0.20–0.33], Fig. 1E), respectively, compared with p16-negativity. However, no prognostic impact of p16-status was found in tumors of non-oropharyngeal origin for either endpoint: LRC (53% vs. 48%, HR: 0.94 [0.63–1.40], Fig. 1B), EFS (28% vs. 28%, HR: 0.94 [0.68–1.30], Fig. 1D), and OS (38% vs. 35%, HR: 0.81 [0.56–1.13], Fig. 1F), respectively. To further investigate whether the impact of p16 would differ by non-OPC sub-site, analysis was also done for larynx and hypopharynx individually. This revealed that p16-status did not impact on outcome in neither tumors of the larynx (LRC

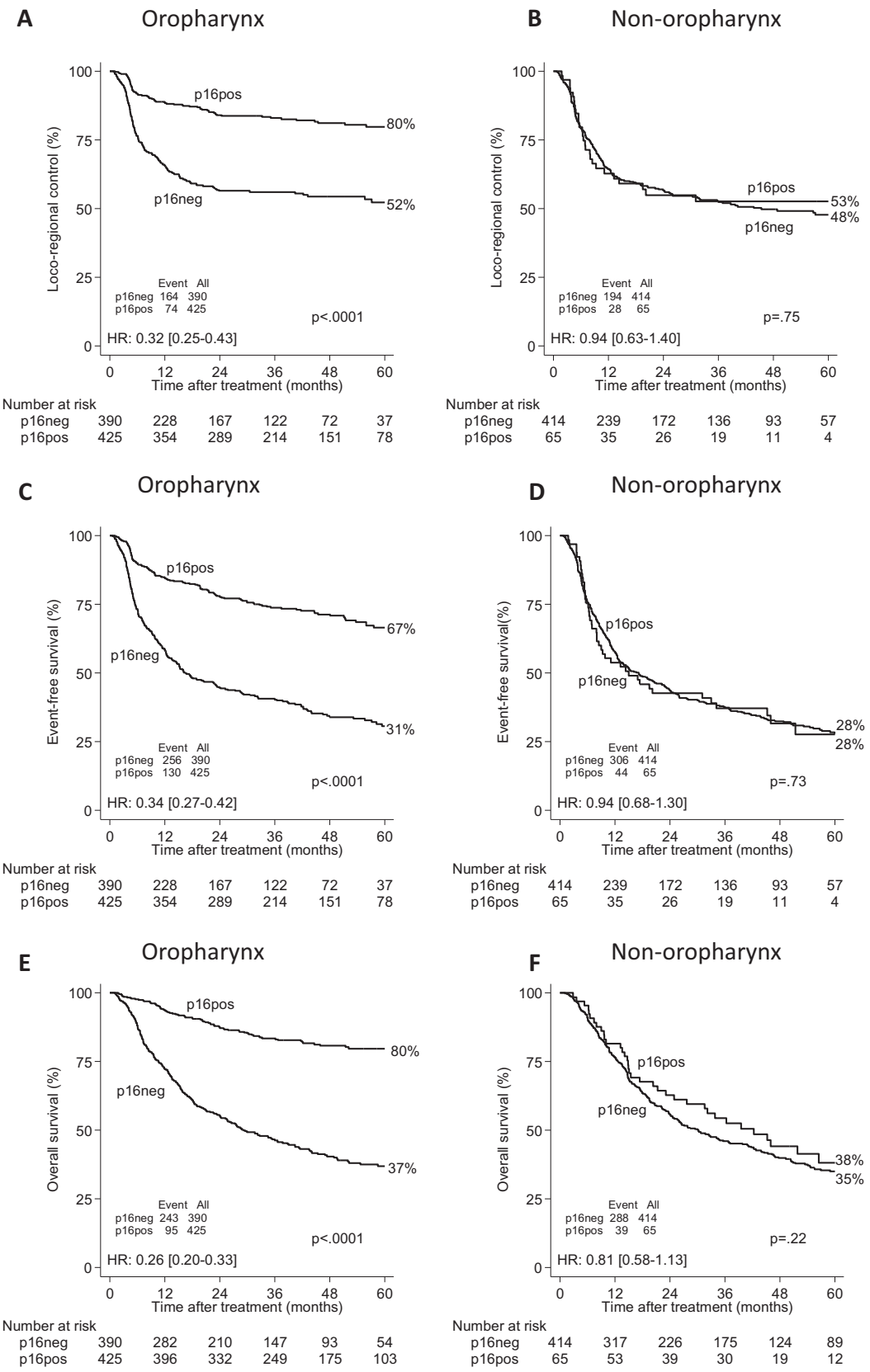

Fig. 1. (A) Actuarial estimated loco-regional tumor control (LRC), (C) event-free survival (EFS) and (E) overall survival (OS) by p16-status in stage III–IV oropharyngeal carcinomas. (B), (D) and (F) illustrate the actuarial estimated LRC, EFS and OS in the group of advanced non-oropharyngeal carcinoma, respectively.

adjusted HR: 1.09 [0.66–1.79], EFS: 0.91 [0.60–1.37] and OS: 0.77 [0.51–1.18]) nor hypopharynx (LRC adjusted HR: 0.86 [0.42–1.78], EFS: 1.09 [0.63–1.89] and OS: 0.74 [0.41–1.31]), respectively.

In the final Cox proportional hazards analysis adjusting for age (<60 years vs. >60 years), gender (female vs. male), T-size (T1–2 vs. T3–4), lymphnode involvement (N0 vs. N+), hypoxic modification

(Nimorazole vs. no Nimorazole), fractionation schedule (6fx/week vs. 5fx/week) and chemotherapy (chemotherapy vs. no chemotherapy), p16-status remained a statistical significant independent prognostic factor in the group of patients with OPC (locoregional failure: HR: 0.43 [0.32–0.57], event-free death: HR: 0.44 [0.35–0.56] and overall death: HR: 0.38 [0.29–0.49]), whereas no prognostic impact of p16-status could be demonstrated in the group of patients with non-OPC: (locoregional failure: HR: 1.13 [0.75–1.70], event-free death: HR: 1.06 [0.76–1.47] and overall death: HR: 0.82 [0.59–1.16]), Table 2. We did an additional analysis involving all 1294 patients including an assessment of the interaction between p16-status and tumor site (OPC vs. non-OPC) for all three endpoints in question. Table 2 shows that p16-positivity interacted significantly ($p < 0.0001$) with oropharyngeal tumor origin only. Thus, outcome was significantly better for all endpoints in patients with p16-positive OPC compared with any other patient group. Moreover, outcomes did not differ significantly between the remaining three groups: p16-neg OPC, p16-pos non-OPC and p16-neg non-OPC.

Discussion

Knowing that HPV-associated p16-positve HNSCC is predominantly characterized by oropharyngeal origin and advanced disease stage, we addressed the question of the potential prognostic impact of p16-status in non-OPC in a selected cohort of patients with stage III–IV disease. Moreover, we only included patients who were treated with primary curatively intended (chemo)radiotherapy, deliberately excluding patients with oral cavity cancer. Within this cohort we confirmed the well-known highly significant independent influence of tumor p16-expression on loco-regional control and survival for patients with advanced OPC. In contrast we could not demonstrate a similar prognostic impact in advanced tumors of the larynx and hypopharynx where radiotherapy-outcome did not differ by tumor p16-status. As opposed to our findings, Chung et al. recently found significant outcome differences within a cohort of non-OPC where the p16-positive group was found to have significant better outcome than the p16-negative non-OPC. [23]. A possible explanation for the diverging results between the two studies might be found in the rather distinct differences between the compositions of the study-cohorts examined. In the RTOG trial one third of the p16-positive tumors were carcinomas of the oral cavity treated with primary surgery followed by postoperative chemoradiation and Cetuximab in the phase II RTOG 0234-trial [28], whereas our analyses only comprised larynx and pharynx cancer treated with primary (chemo)radiotherapy.

Tobacco-smoking negatively affects outcome for head and neck cancer patients treated with radiotherapy [29]. Moreover, it has been shown that also for p16-positive OPC an accumulated smoking history of more or less than 10 packyears independently

Table 2
Multivariable analysis for all patients and stratified by tumor-site.

Endpoint/variable	Oropharynx HR [95% CI][b]	Non-oropharynx HR [95% CI][b]	All patients[a] HR [95% CI][b]
Loco-regional failure			
Age <60 years vs. >60 years	0.89 [0.68–1.15]	1.31 [1.00–1.71]	1.06 [0.88–1.28]
Female vs. male	0.72 [0.52–1.01]	0.51 [0.35–0.76]	0.64 [0.50–0.83]
T1–2 vs. T3–4	0.52 [0.40–0.69]	0.64 [0.47–0.88]	0.57 [0.46–0.70]
N0 vs. N+	0.41 [0.27–0.65]	0.46 [0.33–0.64]	0.44 [0.34–0.57]
p16pos vs. p16neg	0.43 [0.32–0.57]	1.13 [0.75–1.70]	
Nim vs. no nim	0.78 [0.52–1.15]	0.72 [0.50–1.03]	0.74 [0.57–0.97]
6fx/week vs. 5fx/week	0.42 [0.31–0.58]	0.59 [0.43–0.82]	0.50 [0.40–0.63]
Chemo vs. no chemo	0.58 [0.39–0.87]	0.32 [0.17–0.58]	0.47 [0.34–0.65]
p16pos OPC			0.41 [0.31–0.55]
p16pos non-OPC			1.12 [0.75–1.68]
p16neg OPC			0.95 [0.77–1.18]
p16neg non-OPC			1 (Ref)
Event-free death			
Age <60 years vs. >60 years	0.78 [0.63–0.96]	0.99 [0.80–1.22]	0.86 [0.74–0.99]
Female vs. male	0.84 [0.65–1.08]	0.6 0 [0.45–0.81]	0.76 [0.63–0.91]
T1–2 vs. T3–4	0.47 [0.38–0.59]	0.60 [0.46–0.77]	0.52 [0.44–0.62]
N0 vs. N+	0.52 [0.38–0.72]	0.47 [0.36–0.61]	0.48 [0.39–0.59]
p16pos vs. p16neg	0.44 [0.35–0.56]	1.06 [0.76–1.47]	
Nim vs. no nim	0.69 [0.50–0.97]	0.95 [0.69–1.29]	0.82 [0.66–1.03]
6Fx/week vs. 5Fx/week	0.57 [0.44–0.74]	0.65 [0.50–0.84]	0.60 [0.51–0.73]
Chemo vs. no chemo	0.57 [0.42–0.77]	0.49 [0.32–0.72]	0.53 [0.42–0.68]
p16pos OPC			0.41 [0.33–0.51]
p16pos non-OPC			1.06 [0.77–1.47]
p16neg OPC			0.97 [0.82–1.15]
p16neg non-OPC			1 (Ref)
Overall death			
Age <60 years vs. >60 years	0.72 [0.58–0.90]	0.85 [0.68–1.06]	0.77 [0.66–0.90]
Female vs. male	0.84 [0.64–1.09]	0.65 [0.49–0.88]	0.79 [0.65–0.95]
T1–2 vs. T3–4	0.43 [0.34–0.54]	0.62 [0.48–0.81]	0.50 [0.42–0.60]
N0 vs. N+	0.48 [0.35–0.67]	0.44 [0.33–0.58]	0.43 [0.35–0.53]
p16pos vs. p16neg	0.38 [0.29–0.49]	0.82 [0.59–1.16]	
Nim vs. no nim	0.78 [0.56–1.09]	1.25 [0.90–1.73]	1.03 [0.81–1.29]
6Fx/week vs. 5Fx/week	0.60 [0.46–0.78]	0.69 [0.53–0.90]	0.64 [0.53–0.77]
Chemo vs. no chemo	0.36 [0.24–0.53]	0.52 [0.34–0.79]	0.42 [0.31–0.55]
p16pos OPC			0.35 [0.27–0.44]
p16pos non-OPC			0.84 [0.60–1.18]
p16neg OPC			1.00 [0.85–1.21]
p16neg non-OPC			1 (Ref)

Abbreviation: HR, hazard ratio.
[a] Analysis included an assessment of the interaction between p16-status and tumor-site.
[b] Estimates <1 is in favor of the first-mentioned stratum.

influences outcome [18] indicating the existence of a separate group of tumors with mixed aetiology (tobacco and HPV) of importance for clinical outcome. We did not investigate the impact of smoking in the present study. However based on a previous study involving DAHANCA patients [29] we know, that the vast majority of patients with non-OPC were smokers and as such the possibility that differences in smoking habits within the group of non-OPC affect the results seems modest. In the group of OPC, on the other hand, it cannot be ruled out, that there are some "never-smokers" (<10 packyears) among p16-positive patients that further lift their outcome and render the difference in prognosis between p16-positive and p16-negative OPC patients even more pronounced.

The reasons for the apparent site-specific difference in the prognostic impact of p16-expression observed in the present study remain unsolved. Observations from preclinical studies indicate that the enhanced in vitro radiosensitivity of HPV/p16-positive cell lines derived from OPC is linked to the individual tumor cell [20,21] and impaired DSB repair capacity of the tumor cells has been suggested to be the predominant mechanism behind the pronounced radiosensitivity [21]. Whether HPV/p16-positive tumor cells from non-oropharyngeal sites also possess such enhanced in vitro radiosensitivity remains to be elucidated. Moreover, one might speculate that the improved radiotherapy-response observed for HPV/p16-positive OPC may also be associated with the proximity of these tumors to lymphoid tissue and that interplay between the immune-system and the viral antigens may contribute to the enhanced radiosensitivity. Naturally, these hypotheses are far from consolidated and warrant further investigation.

Investigation of the frequency and site-specific distribution of p16-expression (diffuse staining in >70% tumor cell) in this large cohort of advanced carcinoma of the pharynx and larynx revealed that p16-positivity is significantly more frequent in the oropharynx than in the larynx and hypopharynx, and that p16-positivity at the two last-mentioned sites is a relatively rare event. The use of p16-IHC as a marker of infection with HPV in OPC is widely used in clinical trials based on the high concordance between this method and various other HPV detection methods, including type specific HPV-DNA detection by situ hybridization (ISH) [30]. Outside oropharynx the correlation is less robust and viral detection rates show considerable variation which in part can be ascribed to tumor-site differences but also the use of diverse detection methods is of importance [7,8,31]. Literature reviews estimate that the prevalence of HPV in larynx cancer is approximately 24%, based on PCR-detection methods [7,8], whereas data on the frequency in hypopharynx is sparse. Using combined HPV-testing with detection of HPV DNA by PCR and HPV E6/E7 RNA by RT-PCR in 222 HNSCCs, Salazar et al. [32] found a frequency of HPV-positivity of 35% in OPC, 29% in hypopharynx cancer and 5% in laryngeal tumors. They found significant prognostic impact of HPV-status in OPC, whereas HPV-status appeared to be of no significance for outcome in non-OPC regardless of the applied treatment modality. Expression of p16 is not restricted to HPV-positive tumors and using this marker alone as a surrogate of HPV-induced carcinogenesis inevitably entails the risk of including some (virally) false-positive results. We found that the frequency of p16-positivity was 14% for both larynx and hypopharynx carcinomas which is in agreement with the findings of Chung et al. [23] who also investigated the correlation between p16-IHC and HPV-16 ISH and found a moderate correlation for tumors of these sites.

The strong and independent prognostic impact of tumor p16-status on radiotherapy outcome in advanced OPC is indisputable. In this study we observed, that the number of p16-positive tumors in the larynx and hypopharynx is relatively rare. Moreover, we did not find any indication that the prognosis for patients with p16-positive larynx and hypopharynx carcinoma differs from that of patients with p16-negative OPC and p16-negative non-OPC. Rather, these patient-groups have a significantly worse outcome to primary radiotherapy than patients with p16-positive OPC, and as such our findings failed to prove the hypothesis that the impact of tumor p16-status also extends to non-OPC. Consequently, our data suggests that patients with p16-positive tumors of the larynx and hypopharynx should be considered candidates for enhanced, multimodality treatment schedules in line with p16-negative HNSCC.

Conflict of interest statement

The authors have nothing to disclose.

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