

ORIGINAL ARTICLE

Human papilloma virus status evaluation and survival description in selected oropharyngeal and laryngeal squamous cell carcinoma patients from Hungary

Eniko Reka Fejer¹, Zoltan Abram¹, Imre Zs Egyed¹, Septimiu Voidazan¹, Erika Toth², Zoltan Szentirmay², Miklos Kasler²

¹University of Medicine and Pharmacy of Targu Mures, Romania; ²National Institute of Oncology, Budapest, Hungary

Summary

Purpose: Many patients with oropharyngeal squamous cell carcinomas do not have any of the traditional risk factors associated with head and neck squamous cell cancers (HNSCC). Epidemiologic and molecular studies have identified human papillomavirus (HPV) as a causative agent, viral tumors presenting a better survival and being important risk factors together with the long established ones, tobacco and alcohol consumption, in head and neck cancers. The purpose of this study was to establish the incidence of HPV-associated HNSCCs, to identify the most frequent HPV type and to evaluate the overall survival and recurrence rates of HPV-positive cases in comparison with HPV-negative HNSCCs.

Methods: A retrospective analysis from the database of the National Institute of Oncology from Budapest was performed and the following parameters were analyzed: age, age at diagnosis, gender, primary tumor location, tumor histopathology, TNM stage, HPV status, date of recurrence, last visit and date of death.

Results: Out of 81 patients with HNSCCs 55 (67.9%) were male and 26 (32.1%) female. HNSCCs were more frequent

in men (2.11:1) and the majority of the patients (81.7%) were diagnosed in advanced stages (TNM III and IV). HPV status was evaluated in nearly half (48.14%) of the patients and HNSCCs were positive for HPV in 43.6% of the cases. These were more frequent in patients over 50 years (76.66%), in men (76.47%) and in oropharyngeal location (94.1%). HPV-16 type was associated with malignancy in 82.35% of the cases. Disease recurrence was more frequent in HPV-negative (31.81%) vs HPV-positive cases (29.41%) and mortality rate was inferior in HPV-positive 33.33% vs negative (38.09%) tumors ($p=0.52$).

Conclusions: In Hungary HNSCCs are more frequent in men than in women. HPV positivity is higher in men vs women and in oropharyngeal vs laryngeal location. Overall survival rate was superior in HPV-positive vs HPV-negative cases. Disease recurrence was more frequent in HPV-negative vs HPV-positive cases.

Key words: head and neck cancer, human papilloma virus, oropharyngeal squamous cell carcinoma, recurrence, survival

Introduction

Oropharyngeal squamous cell carcinoma (OPSCC) is a multifactorial disease connected with social habits, particularly tobacco use and alcohol consumption, long-established as the principal risk factors, both having synergistic effects.

The incidence of tobacco and heavy alcohol consumption-related HNSCCs has decreased in US and worldwide, while OPSCCs show a constant rise, especially the HPV-related ones. HPV-OPSCC most commonly arises from the oropharynx, pri-

marily the base of the tongue and palatine tonsils. In the US, HPV was estimated to account for 16% of OSCCs in early 1980s, yet the prevalence in the most recent studies exceeds 60% [1-3]. The number of new oropharyngeal cancer cases caused by HPV will likely exceed the number of cervical cancer cases by 2020. HPV-positive tumor status significantly improves survival, is associated with smaller primary tumors (T stage) but more advanced nodal stage (N), and more frequent distant metastases (M) in multiple organs [4,5]. Furthermore, HPV-positive OPSCCs respond much better to therapy than HPV-negative ones and other head and neck cancers (5-year disease-specific survival 80 vs 40%) [3,6].

The purpose of this study was to evaluate the HPV status, the HPV positivity in different anatomical subsites, gender distribution, survival and disease free survival in HPV positive and negative tumors.

Methods

A retrospective search of the National Institute of Oncology Budapest database was performed to identify head and neck cancer cases between 2009-2014. Included were squamous cell carcinomas from the oral cavity, pharynx (epi-, meso- and hypopharynx) and larynx. Nasal cavity, maxilla, mandible, other bone, cheek cutaneous or salivary gland malignancies, and non-mucosal sites were excluded. Cases were categorized in two groups as follows: the first group was the oropharyngeal group with cancers from oral cavity and pharynx, and the second was the laryngeal group (general description in Table 1). We selected 81 cases of malignancies and analysed them in relation to age, age at diagnosis, gender, primary tumor location, tumor histopathology, TNM stage, HPV status, p16 protein evaluation, date of recurrence, date of death, and last visit. HPV detection was made on formalin fixed-paraffin embedded material with real time PCR method and subtypes were evaluated by Linear Array HPV genotyping test using controls. In a limited number of cases (N=7) the HPV detection was made using p16 protein evaluation by immunohistochemistry.

Statistics

Statistical analyses were performed using the MedCalc Software, version 12.5.0.0. Data were labelled as nominal or quantitative variables. Nominal variables were characterized by means of frequencies. The frequencies of nominal variables were compared with chi-square test. Qualitative dichotomous data were used to follow the relation between variables, and null hypothesis (H0) was accepted or refused according to p value. If $p < 0.05$ we accepted the alternative hypothe-

Table 1. General description of the analysed groups

Parameters	Patients, N	%
Age groups (years)		
<40	6	7.4
41- 50	13	16.04
51- 60	23	28.39
61-70	24	29.62
71- 80	12	14.81
>80	3	3.7
Gender		
Female	26	32.1
Male	55	67.9
Location		
Oropharynx	63	77.8
Larynx	18	22.2
TNM stage		
I	5	8.3
II	6	10.0
III	18	30.0
IV	31	51.7
HPV positive		
Yes	17	43.6
No	22	56.4
Recurrence		
Yes	30	37.0
No	51	63.0

sis (H1). Survival analysis was made by Kaplan-Meier method with log-rank test, considering tumor specific mortality, non-tumor specific mortality and patients alive. Tumor specific mortality was defined as the time interval between diagnosis and tumor related fatal event, and the non-tumor related deaths were censored. We used status codes 1- for deceased cases and 0- for alive patients. Disease free survival (DFS) was defined as the time interval between successful treatment and disease recurrence. Recurrence was evaluated in months between diagnosis/treatment and evidence of disease evolution. We assessed overall survival rates with 95% confidence intervals at 1, 2, 5 years, median/range survival, hazard ratio (HR) for risk of death using the Mantel-Cox test.

Results

Gender and age groups distribution

Of 81 patients 55 were male (67.9%) and 26 female (32.1%). HPV status was evaluated in 39 (43.6%) patients (9 female;23.1% and 30 male;76.9%). Tumors were HPV positive in 4 women and 13 men. Of 13 patients < 50 years we found 5 HPV-positive cases (38.46%) and of 26 cases in the > 50 years age group we found 12 (46.15%) HPV-positive tumors.

Table 2. HPV type evaluation in oropharynx and larynx

HPV type	Patients	
	N	%
Negative	22	56.4
11	1	2.6
16	14	35.9
73	2	5.1

Location

The locations of head and neck squamous cell carcinomas were as follows: 18 (22.2%) in the larynx and 63 (77.8%) in the oropharynx.

TNM stage

The advanced stages (TNM III and IV) represented 81.7% of assessed cases, while in 18.3% the disease was localized (TNM stage I, II). In 25.92% of the cases the disease stage could not be assessed because patients were diagnosed and treated in non specialized centers, without a clear initial stage assessment.

Histopathological diagnosis

Histopathological results were not available for 4 patients. Seventy-two (93.5%) had squamous cell carcinoma and 5 (6.5%) other types.

HPV positivity

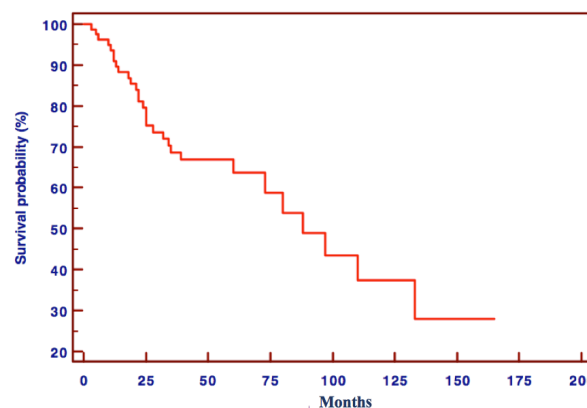
HPV status was evaluated in nearly half (48.14%) of the patients and 43.6% had HPV-positive disease (Table 2). HPV-16 type was associated with malignancy in 82.35% of the cases. These were more frequent in patients over 50 years of age (76.66%), in men (76.47%) and in oropharyngeal location (94.1%).

HPV positivity was noticed in 5.9% of laryngeal and 94.1% of oropharyngeal cancers. ($p=0.0001$) Also, HPV positivity was noticed in 76.47% ($N=13$) of men and 23.52% ($N=4$) of women ($p=0.006$).

P16 protein was evaluated in 7 out of 39 cases. All of them were positive in men and oropharyngeal location.

Recurrence

Thirty patients (37%) developed recurrence and 51 (63%) did not. The median survival in case of recurrence was 66.9 months (range 41.7-92.2) and 90.5 months (range 65.8-115.1) in patients without recurrence. We registered 5 recurrences in HPV-positive tumors (29.41%) and 7 in HPV-

**Figure 1.** Overall survival in Head & Neck squamous cell carcinomas.

negative cancers (31.8%). The median time to recurrence was 17.5 months (range 0-118), and the median time to last visit for those without recurrence was 27.0 months (range 3-158).

Survival

End-of-study survival was evaluated in January 2015. Thirty patients (37%) had died and 51 (63%) were alive with stable disease. Six (7.4%) patients died from causes other than malignancy, 24 (29.6%) died of disease, and 51 (63%) were disease-free. The estimated median overall survival was 90.7 months (range 72.6-108.8). Overall survival rate at one year was 90.9% (95%CI : 87.9-93.9), at 2 years 79.6% (95%CI: 75.6-83.6), and at 5 years 63.7% (95%CI: 57.7-69.7). Median disease-specific survival was 98.9 months (range 79.9-117.8). Disease specific survival rate at one year was 93.0% (95%CI : 90.0-96.0), at 2 years 85.3% (95%CI: 81.3-89.3), and at 5 years 67.7% (95%CI : 61.7-73.7) (Figure 1).

Of the 24 deceased patients 6 (25%) were female and 18 (75%) male. As for disease-specific survival in relation to gender 26.1% of women and 34.6% of men died of cancer ($p=0.16$, HR-1.89, 95%CI : 0.83-4.30) The estimated median survival in women was 97.1 months and 93.04 months in men ($p=0.25$). In women the overall survival rate at one year was 95.7% (95%CI: 91.7-99.7), at 2 years 91.1% (95%CI: 85.1-97.1), and at 5 years 78.7% (95%CI: 69.7-87.7). In men overall survival rate at one year was 91.8% (95%CI: 87.8-95.8), at 2 years 79.8% (95%CI: 73.8-85.8), and at 5 years 62.8% (95%CI: 55.8-68.9).

In younger patients (<50 years), overall survival was 90.9% (95%CI: 82.3-99.6), at 1 year 75.8% (95%CI: 60.2-91.4 at 2 years), while the

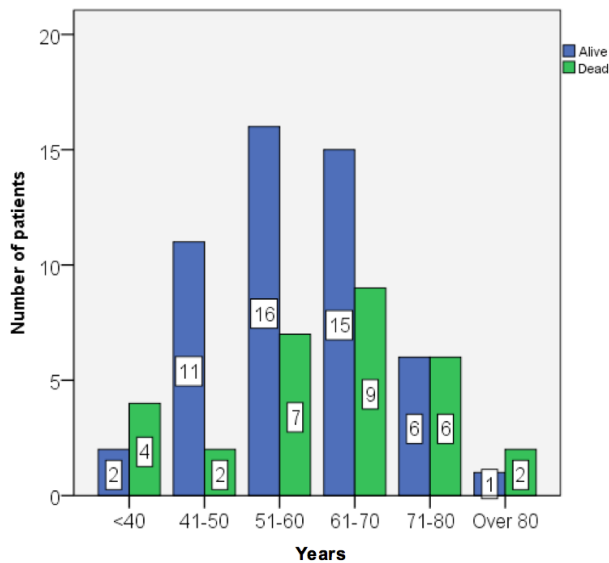


Figure 2. Overall survival in Head & Neck squamous cell carcinomas according to age groups.

highest mortality was registered in the 61-70 year age group (30%) ($p=0.15$; Figure 2).

There were 8 (33.3%) laryngeal and 16 oropharyngeal (66.7%) tumor specific deaths. Half (50%) of 16 cases with laryngeal cancer patients died. In oropharyngeal disease location 27.1% out of 59 patients died (HR-0.80, 95%CI: 0.33-1.94, $p=0.64$) (Figure 3). The estimated median survival was 92.1 months (range 64.4-119.8) for larynx and 105.5 months (range 81.1-129.8) for oropharynx. In larynx the overall survival rate at one year was 100.0% (95%CI: 98.7-100.0), at 2 years 87.5% (95%CI: 79.5-95.5), and at 5 years 66.1% (95%CI: 54.1-78.1). In oropharynx the overall survival rate at one year was 90.9% (95%CI: 87.9-93.9), at 2 years 84.8% (95%CI: 79.8-89.8), and at 5 years 68.3% (95%CI: 61.3-75.3). Mortality in relation to HPV was 38.09% in HPV-negative cases and 33.33% in HPV-positive cases ($p=0.52$).

Twenty-seven patients had disease recurrence, and 10 (37%) of them died. Out of 48 patients without recurrence 14 (29.2%) died of causes other than cancer (Figure 4). The estimated median survival in recurrent cases was 66.9 months (range 41.7-92.2) and 90.5 months (range 65.8-115.1) for those without recurrence ($p=0.15$). Overall survival rate in cases with recurrence at one year was 87.4% (95%CI: 87.33-87.46), at 2 years 64.7% (95%CI: 64.59- 64.81), and at 5 years 55.5% (95%CI: 55.38-55.62). Overall survival rate in cases without recurrence at one year was 91.1% (95%CI: 87.1-95.1), at 2 years 85.8% (95%CI: 80.5-90.8), and at 5 years 69.4% (95%CI: 62.4-76.4).

Recurrence was more frequent in HPV-nega-

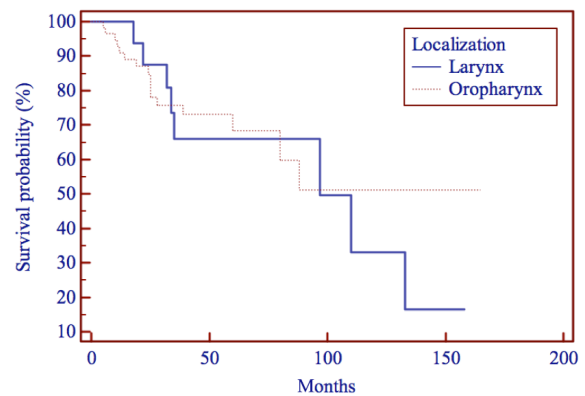


Figure 3. Disease-specific survival in Head & Neck squamous cell carcinomas according to primary tumor location ($p=0.64$).

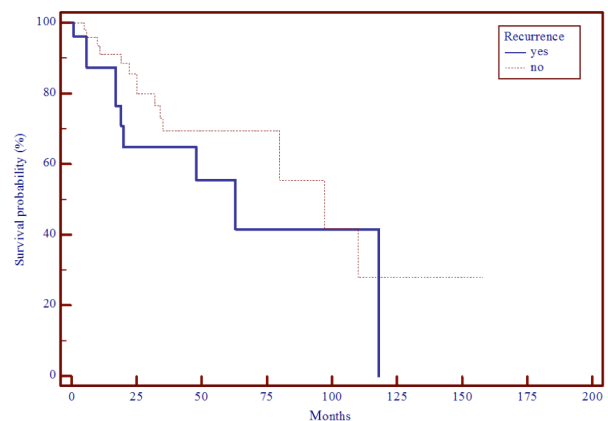


Figure 4. Tumor specific survival in relation to recurrence in Head and Neck squamous cell carcinomas.

tive cases (75 vs 50% HPV-positive) but without statistically significant difference ($p=0.61$).

Discussion

The role of high risk human papillomaviruses (HR-HPV) in the pathogenesis of HNSCCs was first suspected in 1983 when histopathological features consistent with HPV infection were identified in oral cancers. Since then, strong evidence has confirmed this hypothesis and in 2009 the International Agency for Research on Cancer recognized HPV16 as a causal agent in a subset of OPSCCs.

Demographic analysis of our results of the HPV-positive patients was consistent with findings reported in the literature [3,7].

Age groups

HPV-positive tumors are described as more frequent in younger patients [3,7], they lack risk factors such as smoking and alcohol, and are more frequent in men than in women [3]. Our study was realized from a database which did not contain smoking and alcohol consumption related data, and, therefore, didn't confirm these findings.

HPV transmission is primarily by sexual contact and men are more likely than women to have an oral HPV infection because the female genital mucosa has a higher HPV viral load. Younger age of sexual debut, promiscuous sexual habits and increase in the number of sexual partners contribute to a rise in oral/oropharyngeal HPV exposures [8]. Of our patients, 67.9% were male and 32.1% female; 13 (76.46%) men and 4 (23.52%) women had HPV-positive tumors ($p=0.006$). This finding is in agreement with recent studies estimating the prevalence of oral HPV in a healthy population in North America, which showed a higher HPV prevalence in men than in women [9-11]. Analysis by sex did not show any significant differences in HPV DNA prevalence, even though men have 2-fold higher incidence of HNSCCs than women.

Location

The European Commission funded a global study which estimated HPV DNA prevalence in HNSCCs. The reported incidence was 45.8% for oropharynx, 22.1% for larynx (including hypopharynx), and 24.2% for oral cavity [8]. In our study HPV positivity wasn't evaluated in half of the patients, and this may have influenced our results. The European Commission's evaluation of oral cavity and oropharynx together (70.0%) stands for our oropharyngeal location group, in which we included the hypopharynx too. This fact might explain why laryngeal carcinomas have higher HPV positivity. Many studies have shown that HPV-positive OPSCC are predominantly located in the tonsillar complex and base of the tongue [7,13,15]. Our results differ from the multinational study conducted by Mehanna et al. [1] in which they found 72.2% HPV positivity between 2005 and 2009. Another possible explanation might be the high incidence of smoking and alcohol consumption and bad oral hygiene-related tumors which show high incidence in our region [14]. In the past 20 years, growing evidence for the involvement of HPV in HNSCCs has been collected [1-3], especially in developed countries, where smoking cessation and educational

programs helped reduce the OPSCCs incidence [15,16]. However we identified the same results for HPV incidence in OPSCCs in an Italian study, in which the overall incidence of oral HPV was 25.3%, independent of the sampling procedures (biopsy or brushing) [8,17], which coincides with 25.39% for oropharyngeal location in our study. Another study performed by Stojan et al. in Slovenia found 20% HPV associated oropharyngeal cancers between 2007-2008 and a 32% positivity between 2011-2012 [18].

TNM stage

TNM description was found in 60 cases which were distributed in early stage (stage I and II, 18.3%) and locally advanced tumors (stage III and IV, 81.7%). In the literature [4,5] we found descriptions about T4 HPV-positive tumors, more likely associated with bilateral and/or contralateral disease (stage III and IV) compared to smaller tumors. Although they represent advanced stage, HPV-positive OPSCCs have a better response to therapy and longer overall survival [16]. An epidemiological evaluation found HPV-positive OPSCC to associate more likely with small primary tumors and more extensive nodal disease [7].

HPV positivity

In HPV-positive OPSCCs, HPV16 accounted for 90% [3]. HPV DNA was present in 45 histopathological samples (88.2%). Twenty-seven (52.9%) of them hosted 1 or more HR HPV genotypes [19]. A non-significant increased overall HPV DNA prevalence in men compared with women was noted in OPSCC.

Despite presentation with advanced nodal disease, survival was improved in patients with HPV-positive OPSCCs compared with patients with HPV-negative OPSCCs, due to lack of p53 mutation [20]. HPV-positive OPSCCs are less likely to develop second primaries than those with HPV-negative OPSCCs [7].

P16 protein – surrogate marker for oncogenicity

In HPV-driven HNSCCs it is widely known that approximately 80% of cancers are related to HPV16 and HPV18 (high risk subtypes) in all sites, particularly in the oropharynx. The viral transcriptional activity is clinically evaluated by a cell surrogate marker- p16INK4a protein- which proves the carcinogenic activity of the virus. The gold standard test to elucidate the oncogenic role of HPV in the tumor is based on E6/E7 mRNA

techniques, but p16INK4a's immunohistochemical detection association with HPV DNA detection is also validated to identify oncogenetically active HPV infection [21]. In our center only 7 from 39 cases were evaluated by this method, all the positive results being confirmed in oropharyngeal location in men.

The growing evidence of HPV involvement in OPSCCs emphasizes the potential benefit of HPV16 and HPV18 targeting prophylactic vaccines in oropharyngeal location in both sexes [22,23].

Disease recurrence

After primary therapy 37% of the patients recurred. The estimated median survival in case of recurrence was 66.9 months and 90.5 months for those without evolution of the disease ($p=0.15$). Overall, recurrence rates were lower in patients with HPV-positive OPSCCs than in patients with HPV-negative OPSCCs [7].

Survival

Twenty-four (29.6%) patients died of cancer, and 51 (63%) were alive at the end of the study. The estimated median survival – 90.7 months – is an interesting finding. With 81% of patients in disease stage III and IV this survival is considered a good result.

Tumor-specific survival related to gender (26.1% in women vs 34.6% in men) did not show significant difference (HR-1.89, 95%CI 0.83-4.30, $p=0.16$) and the estimated median survival in women was longer (97.1 vs 93.04 months, $p=0.25$).

Tumor-specific survival showed no differences according to the different primary tumor locations (HR-0.80, 95%CI 0.33-1.94) and the estimated median survival in oropharyngeal primary was longer (105.5 vs 92.1 months for larynx $p=0.64$). The higher HPV prevalence at this site could explain the better prognosis of HPV-driven cancers.

The estimated median survival in case of recurrence was 66.9 months and 90.5 months for those without recurrence ($p=0.15$).

Our study may be affected by the inherent drawbacks of a retrospective study, by the limited number of patients and the low HPV evaluation

rate.

The growing incidence of HPV-driven HNSCCs should encourage researches for more intensive studies and evaluation of this cancer type, despite the region's anatomical diversity. Future optimal therapeutic approaches will be based on the understanding the molecular mechanisms, and prophylactic vaccination in adolescent girls and boys may have a cardinal role in reducing the incidence of this disease.

This is the first study to describe and characterize a Central European region's epidemiology, HPV prevalence in HNSCCs and survival of this rarely evaluated location. Our results from a 5 years' selected material with relatively low number of cases confirm the worldwide distribution and pattern of HPV-positive head and neck squamous cell carcinomas.

Conclusions

1. HNSCCs were more frequent in men (67.9%) than in women (32.1%).
2. The vast majority of patients were diagnosed at an advanced TNM stage (III and IV).
3. HPV status was evaluated in half of the patients.
4. HPV positivity was higher in men (76.41%) vs women (23.53%) and in oropharyngeal vs laryngeal location.
5. HPV16 showed the most frequent association (82.35%) with malignancy.
6. Disease-specific survival rate was superior in oropharynx vs larynx.
7. Overall survival rate in HPV-positive vs HPV-negative cases was superior, but without statistical significance.
8. Disease recurrence was more frequent in HPV-negative vs HPV-positive cases, but without statistical significance.

Acknowledgement

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/133377.

References

- Mehanna H, Beech T, Nicholson T et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer-systematic review and meta-analysis of trends by time and region. *Head Neck* 2012 [Epub 2012/01/24]. doi: <http://dx.doi.org/10.1002/hed.22015>
- Rysera M, McGoff K, Herzog D, Sivakoff D, Myers E. Impact of coverage-dependent marginal costs on optimal HPV vaccination strategies. *EPIDEMICS* 2015;173:1-16. <http://dx.doi.org/10.1016/j.epidem.2015.01.003>
- Dalianis T. Human papillomavirus (HPV) and oropharyngeal squamous cell carcinoma. *Presse Med* 2014;43:429-434.
- Benson E, Li R, Eisele D, Fakhry C. The clinical impact of HPV tumor status upon head and neck squamous cell carcinomas. *Oral Oncol* 2014;50:565-574.
- Sood A, McIlwain W, O'Connell B, Nguyen S, Houlton J, Day T. The association between T-stage and clinical nodal metastasis in HPV-positive oropharyngeal cancer. *Head Neck Med* 2014;35:463-468.
- D'Souza G, Dempsey A. The role of HPV in head and neck cancer and review of the HPV vaccine. *Prev Med* 2011;53:S5-S11.
- Pytynia K, Dahlstrom K, Sturgis E. Epidemiology of HPV-associated oropharyngeal cancer. *Oral Oncol* 2014;50:380-386.
- Ndiaye C, Mena M, Alemany L et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. *Lancet Oncol* 2014;15:1319-1331.
- Saedi B, Razmpa E, Ghalandarabadi M et al. Epidemiology of Oral Cavity Cancers in a Country Located in the Esophageal Cancer Belt: A Case Control Study. *Iran J Otorhinolaryngol* 2012;24:113-118.
- Zivile G, Janina D, Irina M, Saulius C, Raisa S, Aliaksandr Z. Prevalence of human papillomaviruses in patients with head and neck squamous cell carcinoma in Lithuania and Belarus. *J Med Virol* 2014;86:531-535.
- Krüger M, Pabst AM, Walter C et al. The prevalence of human papilloma virus (HPV) infections in oral squamous cell carcinomas: A retrospective analysis of 88 patients and literature overview. *J Cranio-Maxillo-Facial Surg* 2014;42:1506-1514.
- Feller L, Wood NH, Khammissa RA et al. Human papillomavirus-mediated carcinogenesis and HPV-associated oral and oropharyngeal squamous cell carcinoma. *Head Face Med* 2010;6:15.
- Cole L, Polfus L, Peters ES. Examining the Incidence of Human Papillomavirus-Associated Head and Neck Cancers by Race and Ethnicity in the U.S., 1995-2005. *Plos One* 2012;7:3.
- Kásler Miklós. *Az onkológia alapjai (The Basics of Oncology)*. Medicina Kiadó, Budapest, 2011,291-400.
- Fung C, Zhou P, Joyce S et al. Identification of epidermal growth factor receptor (EGFR) genetic variants that modify risk for head and neck squamous cell carcinoma. *Cancer Lett* 2015;357:549-556.
- Burtneess B, Bauman J, Galloway T. Novel targets in HPV-negative head and neck cancer: overcoming resistance to EGFR inhibition. *Lancet Oncol* 2013;14:302-309.
- Termine N, Giovannelli L, Rodolico V, Matranga D, Pannone G, Campisi G. Biopsy vs. brushing: Comparison of two sampling methods for the detection of HPV-DNA in squamous cell carcinoma of the oral cavity. *Oral Oncol* 2012;48:870-875.
- Strojan P, Zadnik V, Sifrer R et al. Incidence trends in head and neck squamous cell carcinoma in Slovenia, 1983- 2009: role of human papillomavirus infection. *Eur Arch Otorhinolaryngol* 2015;272:3805-3814.
- Morbini P, Dal Bello B, Alberizzi P et al. Oral HPV infection and persistence in patients with head and neck cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013;116:474-484.
- Oliveira CM, Soares CL, Pinto PL et al. High-risk human papillomavirus (HPV) is not associated with p53 and bcl-2 expression in oral squamous cell carcinomas. *Auris Nasus Larynx* 2009;36:450-456.
- Bussu F, Sali M, Gallus R et al. Human papillomavirus (HPV) infection in squamous cell carcinomas arising from the oropharynx: detection of HPV DNA and p16 immunohistochemistry as diagnostic and prognostic indicators. *Int J Radiat Oncol Biol Phys* 2014;89:1115-1120.
- Hildesheim A, Wacholder S, Catteau G, Struyf F, Dubin G, Herrero R. Efficacy of the HPV-16/18 vaccine: Final according to protocol results from the blinded phase of the randomized Costa Rica HPV-16/18vaccine trial. *Vaccine* 2014;32:5087-5097.
- Herrero R, Quint W, Hildesheim A et al. Reduced prevalence of oral Human Papillomavirus (HPV) 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. *PLoS One* 2013;8(7):e68329. doi:10.1371/journal.pone.0068329