doi:10.4149/neo_2016_013

Human papillomavirus and Epstein-Barr virus in nasopharyngeal carcinoma in a non-endemic Eastern European population

M. SVAJDLER JR[1,2,3,*], J. KASPIRKOVA[1,2], R. MEZENCEV[4], J. LACO[5], T. TORDAY[6], P. DUBINSKY[7], L. STRAKA[8], O. ONDIC[1,2], M. MICHAL[1,2], A. SKALOVA[1,2]

[1]Sikl's Department of Pathology, Charles University in Prague, The Faculty of Medicine and Faculty Hospital in Pilsen, Czech Republic; [2]Biopticka laborator, s.r.o., Pilsen, Czech Republic; [3]Department of Pathology, Louis Pasteur University Hospital, Kosice, Slovakia; [4]Integrated Cancer Research Center, School of Biology and Parker H. Petit Institute of Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA, USA; [5]The Fingerland Department of Pathology, Charles University in Prague, The Faculty of Medicine and University Hospital in Hradec Kralove, Czech Republic; [6]Medicyt s.r.o., Kosice, Slovakia; [7]Department of Radiation Oncology, Oncology Institute, Kosice, Slovakia; [8]Klinicka patologia Presov, s.r.o., Presov, Slovakia

**Correspondence: svajdler@yahoo.com*

Received June 16, 2015 / Accepted August 10, 2015

Nasopharyngeal carcinoma (NPC) is a rare malignancy in the Czech Republic and Slovakia, with the standardized incidence rate of < 1:100000 person-years. Viral status of NPC in these non-endemic Eastern European regions is currently unknown. In a retrospective study, we evaluated the presence of EBV and HPV in 62 NPC cases. EBV status was determined by the use of in situ hybridization (ISH) for EBV encoded small RNA 1 (EBER1). HPV status was examined with p16 immunohistochemistry, DNA ISH and DNA polymerase chain reaction. Sixty-one studied cases showed non-keratinizing morphology and one was keratinizing squamous cell carcinoma. Only one NPC with non-keratinizing morphology was scored as p16-positive (nuclear and cytoplasmic staining ≥ 70% of tumor cells). This case was positive for high-risk HPV by ISH and the DNA PCR confirmed the presence of HPV18 type. At the same time, this case was found negative for EBV. Remaining sixty-one cases that were scored as p16-negative were all found HPV-negative by ISH and the DNA PCR. EBV was detected in 85.5% (53/62) of cases and 9 cases were EBV-negative, including the case of keratinizing NPC. In contrast with previous reports on the prevalence of EBV-positivity in Caucasian patients with NPC, the majority of patients coming from this non-endemic region show EBV-positivity; therefore, they may be candidates for novel EBV-targeting therapies. Conversely, HPV-positive NPC is very rare and HPV does not seem to play a significant role in the etiopathogenesis of NPC in these Eastern European populations.

Key words: squamous cell carcinoma, nasopharyngeal carcinoma, nasopharynx, human papillomavirus, Epstein-Barr virus

Nasopharyngeal carcinoma (NPC) is a very frequent cancer type in some parts of the world, such as Southern China, South-East Asia, Middle East/North Africa and the Arctic, with the annual incidence in some regions up to 1:4000 [1-4]. Epstein-Barr virus (EBV) plays an important role in the pathogenesis of NPC, and is reported to be present in 80-100% of cases from endemic regions, especially in the non-keratinizing subtype [4-6]. Other factors implicated in the carcinogenesis of NPC are environmental carcinogens such as nitrosamines and polycyclic hydrocarbons in salt-preserved food, smoking, cooking and working under poor ventilation, as well as nasal oils, balms and herbal medicines, chemical fumes, dusts and formaldehyde exposure. Some HLA (Humal Leukocyte Antigen) haplotypes are associated with increased risk of developing NPC [1,2,4,5].

In contrast to endemic regions, NPC is rare in Western populations and non-endemic regions, with the annual incidence of < 1:100000 [4]. The strict association between NPC and EBV is not maintained in patients from the low-incidence regions and NPC of the keratinizing subtype [4]. Moreover, several studies from both endemic and non-endemic regions suggested that a significant number of NPC may be related to high risk human papillomavirus (HPV) [7-24]. However, highly variable rates of HPV-positivity in NPC have been found, dependent on the ethnicity of the study population and methods used for HPV detection.

NPC is a highly radiosensitive tumor and radiotherapy remains the standard treatment for all stages of non-disseminated disease [25]; nevertheless, more than 30% of patients relapse after primary treatment with radiotherapy or chemoradiotherapy with either loco-regional recurrence or distant metastases. Chemotherapy is given concurrently with radiotherapy for the curative treatment of locally advanced disease, while palliative chemotherapy is given for metastatic nasopharyngeal carcinoma. The overall survival after recurrence is usually poor and disease control is often associated with radiation- or chemotherapy-related toxicities [25, 26]. The presence of EBV in all tumor cells provides unique opportunities for virus-targeted therapies, which may improve survival and reduce the toxicities. Because prognosis and future therapeutic and preventive approaches may differ considerably among EBV-positive NPC (e.g. EBV-targeted immunotherapy or epigenetic therapy, [27-28]), HPV-positive NPC (HPV vaccination)) and EBV/HPV-negative NPC, the viral status of NPC, as assessed by histopathology, may become more important.

NPC is a rare malignancy in the Czech Republic and Slovakia, with the standardized incidence rate of 0.2 – 0.8/100000 person-years [29,30]. Viral status of NPC in these non-endemic Eastern European regions is currently unknown. In this retrospective study, we evaluated the presence of EBV and HPV in 62 NPC cases. We used p16 immunohistochemistry, DNA in situ hybridization (ISH) and DNA polymerase chain reaction (PCR) for the detection of the biologically relevant HPV infection, and the small RNA ISH for the EBV detection, respectively. In addition, we performed meta-analysis separately for proportion of EBV-positive cases, PCR-detected HPV-positive cases and p16-detected HPV-positive cases in the Caucasian patients with NPC to compare these integrated results with our results and to find out whether different diagnostic methods may play a role in the observed differences among proportions of HPV-positive NPC cases reported in different studies.

Patients and methods

Patients and tissue specimens. 62 patients with primary NPC diagnosed between years 1997 and 2014 were retrieved from the pathology files of two tertiary community hospitals and a large private pathology laboratory, all covering a region of eastern Slovakia (41 cases), tertiary community hospital in Hradec Kralove, Czech Republic (19 cases), and consultation files of the Salivary Gland Tumor Registry at Bioptical Laboratory Plzen, Czech Republic (2 cases). Hematoxylin-eosin slides were reviewed to confirm the diagnosis of NPC and to evaluate the histologic features. The tumors were categorized into keratinizing squamous cell carcinoma (SCC) and nonkeratinizing SCC, which included both differentiated and undifferentiated variants [31]. The basaloid and other rare histological subtypes of NPC were not identified in the study cases.

p16 immunohistochemical staining. For the immunohistochemistry, the most representative paraffin block with tumor tissue was selected in each case and 4 μm tissue sections were stained with the p16 antibody (CINtec® p16 Histology, Ventana) using Ventana Benchmark automated stainer, according to the manufacturer's protocol. Appropriate positive and negative control slides were used. The staining pattern for p16 was classified as positive when showing nuclear and cytoplasmic staining in more than 70% of tumor cells because cutoff ≥ 70% best correlates with the presence of transcriptionally-active HPV in HPV-related oropharyngeal SCC [32].

Polymerase chain reaction. For molecular-genetic studies, genomic DNA was isolated from formalin-fixed, paraffin-embedded tissue using QIAsymphony DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol on QIAsymphony SP device. Special precautions were taken to prevent HPV DNA contamination. The quality of isolated DNA was checked by PCR that amplifies a set of control genes [33].

The HPV DNA detection was performed using multiple PCR primers from the L1, E1, and E6-E7 regions of the HPV genome as previously described [34]. In brief, primers CPSGB, GP5+/GP6+ aiming E1 and L1 region of HPV genome were used for a wide-range detection of high risk and low risk HPV types (HR and LR HPV), and virus type-specific PCR detection of E6-E7 region of six most prevalent HR-HPV types, namely type 16, 18, 31, 33, 35, 45 was used to increase sensitivity of HPV detection and to avoid negative finding due to the possible process of HPV integration into human genome. Furthermore, RHA kit HPV SPF10-LiPA25, version 1 (Bio-medical Products, Rijswijk, The Netherlands) was run in order to reveal possible multiple HPV type.

In situ hybridization. In situ hybridization for HPV DNA detection (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66) was performed according to the manufacturer's protocol using the BenchMark automated slide staining system (Ventana Medical System) and the INFORM HPV III Family 16 Probe (B) set and iViewBlue plus detection kit (Ventana, Roche, Basel, Switzerland). Punctate or diffuse nuclear staining was scored as positive.

EBV in situ hybridization was run on the BenchMark automated slide staining system (Ventana Medical System) using the INFORM EBER fluorescein-labeled oligonucleotide probe and iViewBlue detection kit (Ventana, Roche, Basel, Switzerland) in order to detect the early RNA transcript of Epstein-Barr virus. Diffuse nuclear staining was scored as positive.

Statistical analysis. Meta-analysis search strategy, selection criteria and execution: Overall proportion of EBV- or HPV-positive nasopharyngeal carcinoma cases in Caucasian patients was estimated from previously reported studies by meta-analysis using the fixed effects and random effects models. HPV-positivity defined by PCR and ISH/IHC for p16 were analyzed by separate meta-analyses. Details on meta-analyses are available in the supplementary file.

Statistical significance of differences in HPV-prevalence and EBV-prevalence between male and female patients was

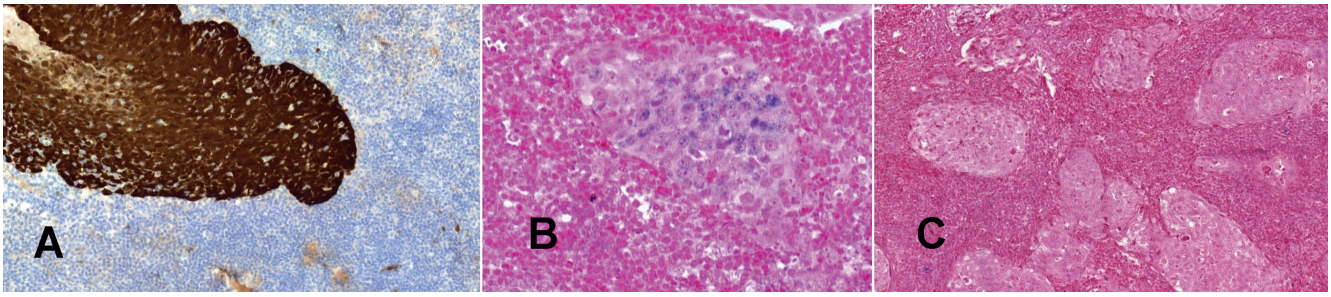


Figure 1. Single case of HPV-positive NPC showed diffuse and strong nuclear and cytoplasmic p16 positivity (A), punctate nuclear staining with HPV DNA in situ hybridization indicating the presence of viral intergration (B), and was EBV negative by RNA in situ hybridization (C).

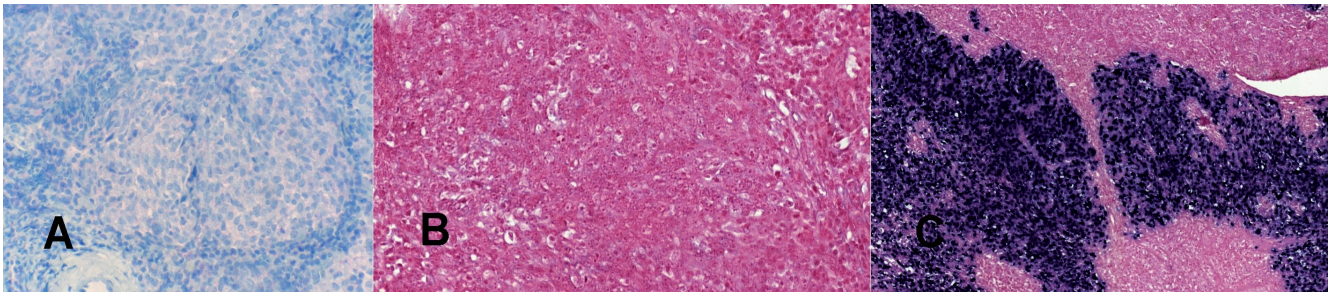


Figure 2. Representative case of p16-negative (A), HPV-negative (B), and EBV RNA-positive NPC (C).

evaluated by Fisher's exact test (Table 1). Statistical significance of differences in mean ages between male and female patients and between EBV-positive and EBV-negative patients was evaluated by Welch's t-test. All reported p-values are two-tailed and differences were considered significant if $p<0.05$.

Results

The study group consisted of 13 females and 49 males, all of Eastern European Caucasian origin (Table 1). They were 46 to 81 years old with the mean age at the time of diagnosis 64.2 years (median, 63 years). Of the 62 NPC cases included in the study, 61 (98.4%) showed non-keratinizing morphology and one (1.6%) was keratinizing SCC. Immunohistochemically, at diagnostic threshold of p16-positivity in ≥70% of tumor cells, only one case (1.6%) was scored as p16-positive (Figure 1a). In this case, high-risk HPV was detected by ISH cocktail probe (Figure 1b) and DNA PCR confirmed the presence of HPV18 type. This HPV-positive NPC occurred in a 41-year-old woman, showed non-keratinizing morphology, and did not show tumor involvement of the oropharynx on the review of the pre-treatment imaging studies.

Remaining 61 cases were scored as p16-negative (Figure 2a), including three cases with weak and focal p16 expression (<70% of tumor cells). All p16-negative cases (including the single case of keratinizing NPC) were HPV-negative by the means of PCR and ISH (Figure 2b).

EBER in situ hybridization was diffusely and strongly positive in the majority of neoplastic cells in 85.5% (53/62) of cases (Figure 2c). The HPV-positive case and the single case of keratinizing NPC were both EBV-negative. Viral status of all 62 NPC cases is summarized in Table 2.

Male patients with NPC displayed lower mean age, lower proportion of HPV-positivity and higher proportion of EBV-positivity than female patients, but these differences were not statistically significant. Interestingly however, EBV-negative patients tended to be older than EBV-positive patients with mean ages 61.6 years and 50.2 years, respectively, and this difference was statistically significant (p=0.0298).

References addressing both EBV and HPV virus status in nasopharyngeal carcinoma were retrieved from MEDLINE/

Table 1. Demographic and clinicopathological characteristics of 62 NPC cases

	Males	Females	P-value
N	49	13	
Mean Age±SD	45.5±13.6	53.5±18.4	0.18
P16-positive	0	1	
P16-negative	49	12	0.21
HPV ISH-positive	0	1	
HPV ISH-negative	49	12	0.21
EBER-positive	43	10	
EBER-negative	6	3	0.38

PubMed and their summary is presented in Supplementary Table 1. Subsets of these references meeting selection criteria were included to individual meta-analyses (supplementary file). Meta-analysis of 8 studies that reported prevalence of EBV-positivity in NPC in Caucasian patients demonstrated heterogeneity among studies at p-value 0.0228. However, considering the number of studies involved in this meta-analysis, large 95% CI for I^2 quantity (5.41-80.42%), and similar summary proportions obtained by fixed and random effects models, these studies appear to show some consistency and the true proportion of EBV-positivity in Caucasian NPC patients can be estimated as ~42%, which considerably differs from our results (85.5%). Consequently, Eastern European Caucasian population studied in this report appears to be more similar to the populations in NPC endemic regions with respect to the prevalence of EBV-positivity in the NPC.

Meta-analysis of 5 studies that reported prevalence of PCR-detected HPV-positivity and 4 studies that reported prevalence of ISH/p16-detected HPV-positivity in NPC in Caucasian patients both demonstrated considerable heterogeneity among studies with I^2>75% and p-values of 0.0018 and 0.0001 respectively (for details see supplementary file). As a result, true proportions of HPV-positivity cannot be estimated and Caucasian patients with NPC likely form two or more subpopulations that differ in the etiological importance of HPV in nasopharyngeal carcinoma.

Discussion

Although EBV is recognized as a major etiologic agent for almost all NPC of the non-keratinizing subtype, especially in patients from endemic regions, association between NPC and EBV is reportedly not maintained in patients from non-endemic regions and in NPC of the keratinizing subtype [4]. However, our study of a group of NPC patients from non-endemic region suggests strong association between NPC and EBV.

Multiple studies have documented the association between high-risk HPV infection and oropharyngeal squamous cell carcinoma (OP-SCC) and high-risk HPV types are detected in the majority of OP-SCC cases in North America and Europe [35-39]. HPV-positive OP-SCC is currently recognized as a clinically distinct variant of SCC, characterized by younger age of onset, non-smoking history, strong association with oro-genital/oro-anal sexual behavior and, most importantly, significantly better outcome when compared to HPV-negative SCC [40]. Given the superior survival, younger age, and good performance status, ongoing clinical trials for oropharyngeal HPV-associated SCC are focused on the de-intensified therapy [40]. These findings have raised a question whether HPV may also play a role in the etiopathogenesis of NPC. Several studies investigated the relationship between HPV and EBV in endemic and non-endemic NPC [7-17,20-24]. The results of these studies are summarized in Supplementary Table 1. Intriguingly, highly variable rates of HPV-positivity in NPC have been found, ranging from 0% to 100% [8,14,22], dependent on the ethnicity of the study population and methods used for HPV detection [7-17,20-24]. In order to integrate the results of these individual studies and to estimate the overall proportion of HPV-positivity in the NPC we performed meta-analysis separately for PCR-detected and ISH/p16-detected proportions of HPV positivity in the Caucasian NPC patients (for details see the supplementary file). The results of these two meta-analyses imply that overall proportion of HPV-positivity estimated by the random effect model is 36.1% (CI95: 16.9-58.0%) and 31.7% (10.7-57.6%) for PCR- and ISH/p16-defined positivity, respectively. Since both these groups of studies display considerable heterogeneity (I^2 > 75% for both meta-analyses), the results of both meta-analyses consistently suggest the existence of two or more distinct sub-populations in which HPV plays different roles in the etiopathogenesis of NPC. Intriguingly, the proportions of HPV-positive NPC cases identified by different methods are similar, which suggests that different diagnostic methods may not play major role in the observed differences among proportions of HPV-positive NPC cases reported in different studies.

Although HPV-related SCC may be found in non-oropharyngeal head and neck subsites, the incidence of true HPV-associated non-oropharyngeal SCC is reportedly overstated [41]. In a recent review of the literature Isayeva et al. found that the weighted prevalence of HPV DNA positive detection in oral cavity cancers, laryngeal cancers and sinonasal cancer was 20.2%, 23.6% and 29.6%, respectively, with highly variable values reported for particular sites ranging from 0% to 100% [42]. There are various factors contributing to overestimation of the role of HPV in non-oropharyngeal cancers. In some instances, HPV-positive nasopharyngeal tumor may just represent an extension from an oropharyngeal primary [20]. On the other hand, highly sensitive DNA PCR assays that are not able to distinguish biologically relevant from inactive infections or just contamination reportedly tend to overestimate the true proportion of HPV-associated non-oropharyngeal SCC [41]. Consistent with that, when more specific HPV detection strategies coupling the presence of HPV with evidence of its transcriptional activity are applied and tumors involving contiguous subsites are excluded by review of the radiology findings, the true proportion of HPV-associated SCC of the oral cavity, hypopharynx and larynx ranges from 0% to 5.9% [43-47], leaving the sinonasal tract the only site with a high proportion of HPV-related cancers [44,48]. When the above mentioned issues are taken into account, the true proportion

Table 2. Summary of the histopathological subtype and viral status of 62 NPC cases

WHO type	EBV-positive NPC	HPV-positive NPC	EBV/HPV-negative NPC
Keratinizing	0	0	1 (1,6%)
Non-keratinizing	53 (85.5%)	1 (1.6%)	7 (11.3%)

of HPV-related NPC appears to be about 5-30% [21-24] ; however, in the light of the results of our meta-analysis the method of HPV detection seems to be a less important factor behind the differences in previously reported proportions of HPV-positive NPC, and other variables, such as ethnicity may play more significant role.

Our findings suggest that HPV infection does not play a significant role in NPC in Caucasian Eastern European (Czech and Slovak) patient population. This information may be of practical importance in patients with neck metastases of HPV-positive SCC from an unknown origin. The probability that HPV-positive cervical metastasis comes from an occult nasopharyngeal primary is very low. This information is crucial for clincal management of the patient because it improves effectivity in terms of costs and time needed to localize the primary tumor.

Surprisingly, some studies identified co-infection of both HPV in EBV in up to 53% of NPC cases [7,10-13,15-17]. EBV/HPV co-infection was found predominantly in studies using DNA PCR techniques [7,10-12,14,16,17], but also in some ISH studies [13,15,17]. However, the most recent studies using ISH assays or DNA PCR in combination with p16 immunohistochemistry did not find any HPV-positive/EBV-positive case [20-24]. Whether HPV/EBV co-infection plays a role in the pathogenesis of NPC is not clear. Interestingly, Jiang et al. recently reported on HPV/EBV co-infection in oropharyngeal SCC and suggested that HPV/EBV co-infection in cancers associated with lymphoid tissue might have a highly tumorigenic potential [49]. They used RNA ISH and PCR detection for EBV and DNA ISH combined with p16 immunohistochemistry for HPV, respectively, and found HPV/EBV co-infection in 4 of 16 tonsilar cancers and 3 of 15 base of tongue cancers but in none of the normal tissues. They also investigated the effect of HPV and EBV infections on the proliferation and invasiveness of SCC (FaDu) cell line and normal oral keratinocyte (NOK) cell line and found that co-infected cell lines showed a significant increase in invasiveness [49].

What is the prognostic significance of the HPV-positivity in NPC is not well established. Five studies reported so far have assessed the prognostic significance of HPV-positivity in NPC. Huang et al. studied 43Taiwanese patients by PCR and found HPV DNA in 35% of cases. No correlation could be found between HPV status and T classification, N classification, stage, disease recurrence or survival [17].

Robinson et al. screened 67 NPC patients by p16 immunohistochemistry and HPV DNA PCR. All cases with p16 over-expression or positive for HPV by PCR were examined by HPV DNA ISH and they found 11 HPV-positive cases (16%). There was no statistically significant difference in overall survival outcome between patients with HPV-positive and HPV-negative NPC [21].

Lin et al. compared 108 white Americans with a cohort of 86 patients from southern China. Tissue microarrays were constructed from the tumor samples and stained with p16 and HPV was detected by PCR and ISH. No HPV-positive cases were detected in the Chinese cohort. In the American cohort, 5 cases harbored HPV type 16. There was no association between HPV status and overall survival, but white patients with EBV-negative NPC showed a trend toward worse overall survival [22].

In a study by Dogan et al., 90 patients with NPC were examined for the presence of HPV by p16 immunohistochemistry and HPV DNA ISH. Of 9 HPV-positive cases, 3 extended from extra-nasopharyngeal sites and nasopharyngeal origin was confirmed in 6 cases. The overall survival of patients with HPV-positive NPC was not significantly different from that of EBV-positive NPC. However, the OS of patients with EBV-negative and HPV-negative NPC was significantly shorter [23].

Finally, Stenmark et al. studied 61 NPC cases using p16 immunohistochemistry and HPV PCR. Eighteen of 61 cases (30%) were HPV-positive. HPV-positive and EBV/HPV-negative tumors exhibited worse outcomes than did EBV-positive tumors, including decreased overall survival, progression-free survival, and locoregional control [24].

Thus, in contrast to oropharyngeal SCC, HPV-positivity in NPC is not associated with better outcomes. However, the data are quite limited and more studies are needed.

Conclusions

To the best of our knowledge, this is the first study investigating simultaneously HPV and EBV viral status in the nasopharyngeal carcinoma in the Eastern European population. We have detected biologically relevant high risk HPV infection (HPV type 18) in only one NPC patient (1.6%). In contrast, the majority (85.5%) of our patients showed EBV-positivity, and significant minority (12.9%) was HPV/EBV-negative. Our findings may have several practical implications: (i) in contrast to other non-endemic regions, the majority of our NPC is EBV-positive, (ii) these patients may be candidates for novel EBV-targeting therapies, such as immunotherapy or epigenetic therapy [25-28], (iii) disease status of the majority of our patients could be monitored by quantitative measurement of circulating EBV DNA [4], and (iv) the probability that HPV-positive cervical metastasis from an unknown origin comes from an occult nasopharyngeal primary is very low in our population and HPV detection should direct attention to oropharynx.

Supplementary information is available in the online version of the paper.

Acknowledgements: Supported by Grant from Charles University for Postdoctoral Student Research (SVV grant 2015, Nr. 260171)

References

[1] THOMPSON LD. Update on nasopharyngeal carcinoma. Head Neck Pathol 2007; 1: 81–86. http://dx.doi.org/10.1007/s12105-007-0012-7

[2] LIU FF, FRAPPIER L, KIM J, O'SULLIVAN B, HUI A et al. East-West Symposium on nasopharyngeal cancer. Int J Radiat Oncol Biol Phys 2007; 67: 703–708. http://dx.doi.org/10.1016/j.ijrobp.2006.09.014

[3] CARLE LN, KO CC, CASTLE JT. Nasopharyngeal carcinoma. Head Neck Pathol 2012; 6: 364–368. http://dx.doi.org/10.1007/s12105-012-0333-z

[4] PETERSSON F. Nasopharyngeal carcinoma: a review. Semin Diagn Pathol 2015; 32: 54–73. http://dx.doi.org/10.1053/j.semdp.2015.02.021

[5] PEREZ-ORDONEZ B. An update on Epstein-Barr virus and nasopharyngeal carcinogenesis. Head Neck Pathol 2007; 1: 141–145. http://dx.doi.org/10.1007/s12105-007-0020-7

[6] TSAO SW, TSANG CM, TO KF, LO KW. The role of Epstein-Barr virus in epithelial malignancies. J Pathol 2015; 235: 323–333. http://dx.doi.org/10.1002/path.4448

[7] TYAN YS, LIU ST, ONG WR, CHEN ML, SHU CH et al. Detection of Epstein-Barr virus and human papillomavirus in head and neck tumors. J Clin Microbiol 1993; 31: 53–56.

[8] HORDING U, NIELSEN HW, DAUGAARD S, ALBECK H. Human papillomavirus types 11 and 16 detected in nasopharyngeal carcinomas by the polymerase chain reaction. Laryngoscope 1994; 104: 99–102. http://dx.doi.org/10.1288/00005537-199401000-00018

[9] GIANNOUDIS A, ERGAZAKI M, SEGAS J, GIOTAKIS J, ADAMOPOULOS G et al. Detection of Epstein-Barr virus and human papillomavirus in nasopharyngeal carcinoma by the polymerase chain reaction technique. Cancer Lett 1995; 89: 177–181. http://dx.doi.org/10.1016/0304-3835-(94)03667-8

[10] RASSEKH CH, RADY PL, ARANY I, TYRING SK, KNUDSEN S et al. Combined Epstein-Barr virus and human papillomavirus infection in nasopharyngeal carcinoma. Laryngoscope 1998; 108: 362–367. http://dx.doi.org/10.1097/00005537-199803000-00010

[11] PUNWANEY R, BRANDWEIN MS, ZHANG DY, URKEN ML, CHENG R et al. Human papillomavirus may be common within nasopharyngeal carcinoma of Caucasian Americans: investigation of Epstein-Barr virus and human papillomavirus in eastern and western nasopharyngeal carcinoma using ligation-dependent polymerase chain reaction. Head Neck 1999; 21: 21–29. http://dx.doi.org/10.1002/(SICI)1097-0347-(199901)21:1<21::AID-HED3>3.0.CO;2-Z

[12] TUNG YC, LIN KH, CHU PY, HSU CC, KUO WR. Detection of human papilloma virus and Epstein-Barr virus DNA in nasopharyngeal carcinoma by polymerase chain reaction. Kaohsiung J Med Sci1999; 15: 256–262.

[13] MIRZAMANI N, SALEHIAN P, FARHADI M, TEHRAN EA. Detection of EBV and HPV in nasopharyngeal carcinoma by in situ hybridization. Exp Mol Pathol 2006; 81: 231–234. http://dx.doi.org/10.1016/j.yexmp.2006.04.006

[14] MAXWELL JH, KUMAR B, FENG FY, MCHUGH JB, CORDELL KG et al. HPV-positive/p16-positive/EBV- negative nasopharyngeal carcinoma in white North Americans. Head Neck 2010; 32: 562–567.

[15] LO EJ, BELL D, WOO J, LI G, HANNA EY et al. Human papillomavirus and WHO type I nasopharyngeal carcinoma. Laryngoscope 2010; 120: 1990–1997. http://dx.doi.org/10.1002/lary.21089

[16] LAANTRI N, ATTALEB M, KANDIL M, NAJI F, MOUTTAKI T et al. Human papillomavirus detection in moroccan patients with nasopharyngeal carcinoma. Infect Agent Cancer 2011; 6: 3. http://dx.doi.org/10.1186/1750-9378-6-3

[17] HUANG CC, HSIAO JR, YANG MW, WU YH, HSU KF et al. Human papilloma virus detection in neoplastic and non-neoplastic nasopharyngeal tissues in Taiwan. J Clin Pathol 2011; 64: 571–577. http://dx.doi.org/10.1136/jcp.2010.087742

[18] DENG Z, HASEGAWA M, MATAYOSHI S, KIYUNA A, YAMASHITA Y et al. Prevalence and clinical features of human papillomavirus in head and neck squamous cell carcinoma in Okinawa, southern Japan. Eur Arch Otorhinolaryngol 2011; 268: 1625–1631. http://dx.doi.org/10.1007/s00405-011-1515-0

[19] BARWAD A, SOOD S, GUPTA N, RAJWANSHI A, PANDA N et al. Human papilloma virus associated head and neck cancer: a PCR based study. Diagn Cytopathol 2012; 40: 893–897. http://dx.doi.org/10.1002/dc.21667

[20] SINGHI AD, CALIFANO J, WESTRA WH. High-risk human papillomavirus in nasopharyngeal carcinoma.Head Neck 2012; 34: 213–218. http://dx.doi.org/10.1002/hed.21714

[21] ROBINSON M, SUH YE, PALERI V, DEVLIN D, AYAZ B et al. Oncogenic human papillomavirus-associated nasopharyngeal carcinoma: an observational study of correlation with ethnicity, histological subtype and outcome in a UK population. Infect Agent Cancer 2013; 8: 30. http://dx.doi.org/10.1186/1750-9378-8-30

[22] LIN Z, KHONG B, KWOK S, CAO H, WEST RB et al. Human papillomavirus 16 detected in nasopharyngeal carcinomas in white Americans but not in endemic southern Chinese patients. Head Neck 2014; 36: 709–714. http://dx.doi.org/10.1002/hed.23362

[23] DOGAN S, HEDBERG ML, FERRIS RL, RATH TJ, ASSAAD AM et al. Human papillomavirus and Epstein-Barr virus in nasopharyngeal carcinoma in a low-incidence population. Head Neck 2014; 36: 511–516. http://dx.doi.org/10.1002/hed.23318

[24] STENMARK MH, MCHUGH JB, SCHIPPER M, WALLINE HM, KOMARCK C et al. Nonendemic HPV-Positive Nasopharyngeal Carcinoma: Association With Poor Prognosis. Int J Radiat Oncol Biol Phys 2014; 88: 580–588. http://dx.doi.org/10.1016/j.ijrobp.2013.11.246

[25] TSANG J, LEE VH, KWONG DL. Novel therapy for nasopharyngeal carcinoma – Where are we. Oral Oncol. 2014 Sep; 50(9): 798–801. http://dx.doi.org/10.1016/j.oraloncology.2014.01.002

[26] HUTAJULU SH, KURNIANDA J, TAN IB, MIDDELDORP JM. Therapeutic implications of Epstein-Barr virus infection for the treatment of nasopharyngeal carcinoma. Ther Clin Risk Manag 2014; 10: 721–736. http://dx.doi.org/10.2147/TCRM.S47434

[27] LOUIS CU, STRAATHOF K, BOLLARD CM, ENNAMURI S, GERKEN C et al. Adoptive transfer of EBV-specific T cells results in sustained clinical responses in patients with locoregional nasopharyngeal carcinoma. J Immunother 2010; 33: 983–990. http://dx.doi.org/10.1097/CJI.0b013e3181f3cbf4

[28] LI J, CHEN QY, HE J, LI ZL, TANG XF et al. Phase I trial of adoptively transferred tumor-infiltrating lymphocyte immunotherapy following concurrent chemoradiotherapy in patients with locoregionally advanced nasopharyngeal carcinoma. Oncoimmunology 2015; 4: e976507. http://dx.doi.org/10.4161/23723556.2014.976507

[29] DIBA CS, PLESKO I, editors. Cancer incidence in the Slovak Republic 2008. Bratislava: NHIC, 2014.

[30] Cancer Incidence in the Czech Republic, 2011. Praha: ÚZIS ČR, NOR ČR, 2015.

[31] CHAN JKC, BRAY F, MCCARRON P, FOO W, LEE AWM et al. Nasopharyngeal Carcinoma. In: Barnes EL, Eveson JW, Reichart P, Sidranksy D, editors. World Health Organization Classification of Tumours – pathology and genetics of head and neck tumors. Lyon: IARC Press, 2005: 85–97.

[32] GRONHOJ LARSEN C, GYLDENLOVE M, JENSEN DH, THERKILDSEN MH, KISS K ET AL. Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: a systematic review, Br J Cancer 2014; 110: 1587–1594. http://dx.doi.org/10.1038/bjc.2014.42

[33] VAN DONGEN JJ, LANGERAK AW, BRUGGEMANN M, EVANS PA, HUMMEL M et al. Design and standardisation of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98–3936. Leukemia 2003; 17: 2257–2317. http://dx.doi.org/10.1038/sj.leu.2403202

[34] SKALOVA A, KASPIRKOVA J, ANDRLE P, HOSTICKA L, VANECEK T. Human papillomaviruses are not involved in the etiopathogenesis of salivary gland tumors. Cesk Patol 2013; 49: 72–75.

[35] CHATURVEDI AK, ENGELS EA, PFEIFFER RM, HERNANDEZ BY, XIAO W et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011; 29: 4294–4301. http://dx.doi.org/10.1200/JCO.2011.36.4596

[36] TINHOFER I, JOHRENS K, KEILHOLZ U, KAUFMANN A, LEHMANN A et al. Contribution of human papilloma virus to the incidence of squamous cell carcinoma of the head and neck in a European population with high smoking prevalence. Eur J Cancer 2015; 51: 514–521. http://dx.doi.org/10.1016/j.ejca.2014.12.018

[37] GARNAES E, KISS K, ANDERSEN L, THERKILDSEN MH, FRANZMANN MB et al. A high and increasing HPV prevalence in tonsillar cancers in Eastern Denmark, 2000–2010: the largest registry-based study to date. Int J Cancer 2015; 136: 2196–2203. http://dx.doi.org/10.1002/ijc.29254

[38] ABOGUNRIN S, DI TANNA GL, KEEPING S, CARROLL S, IHEANACHO I. Prevalence of human papillomavirus in head and neck cancers in European populations: a meta-analysis. BMC Cancer 2014; 14: 968. http://dx.doi.org/10.1186/1471-2407-14-968

[39] LACO J, VOSMIKOVA H, NOVAKOVA V, CELAKOVSKY P, DOLEZALOVA H et al. The role of high-risk human papillomavirus infection in oral and oropharyngeal squamous cell carcinoma in non-smoking and non-drinking patients: a clinicopathological and molecular study of 46 cases. Virchows Arch 2011; 458: 179–187. http://dx.doi.org/10.1007/s00428-010-1037-y

[40] CHATURVEDI AK. Epidemiology and clinical aspects of HPV in head and neck cancers. Head Neck Pathol 2012; 6: Suppl 1 S16–24. http://dx.doi.org/10.1007/s12105-012-0377-0

[41] WESTRA WH. The pathology of HPV-related head and neck cancer: implications for the diagnostic pathologist. Semin Diagn Pathol 2015; 32: 42–53. http://dx.doi.org/10.1053/j.semdp.2015.02.023

[42] ISAYEVA T, LI Y, MASWAHU D, BRANDWEIN-GENSLER M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review, Head Neck Pathol 2012; 6: Suppl 1 S104–120. http://dx.doi.org/10.1007/s12105-012-0368-1

[43] LEWIS JR JS, UKPO OC, MA XJ, FLANAGAN JJ, LUO Y et al. Transcriptionally-active high-risk human papillomavirus is rare in oral cavity and laryngeal/hypopharyngeal squamous cell carcinomas--a tissue microarray study utilizing E6/E7 mRNA in situ hybridization. Histopathology 2012; 60: 982–991. http://dx.doi.org/10.1111/j.1365-2559.2011.04169.x

[44] BISHOP JA, MA XJ, WANG H, LUO Y, ILLEI PB et al. Detection of transcriptionally active high-risk HPV in patients with head and neck squamous cell carcinoma as visualized by a novel E6/E7 mRNA in situ hybridization method. Am J Surg Pathol 2012; 36: 1874–1882. http://dx.doi.org/10.1097/PAS.0b013e318265fb2b

[45] CHERNOCK RD, WANG X, GAO G, LEWIS JR JS, ZHANG Q et al. Detection and significance of human papillomavirus, CDKN2A(p16) and CDKN1A(p21) expression in squamous cell carcinoma of the larynx. Mod Pathol 2013; 26: 223–231. http://dx.doi.org/10.1038/modpathol.2012.159

[46] LINGEN MW, XIAO W, SCHMITT A, JIANG B, PICKARD R et al. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol 2013; 49: 1–8. http://dx.doi.org/10.1016/j.oraloncology.2012.07.002

[47] POLING JS, MA XJ, BUI S, LUO Y, LI R et al. Human papillomavirus (HPV) status of non-tobacco related squamous cell carcinomas of the lateral tongue. Oral Oncol 2014; 50: 306–310. http://dx.doi.org/10.1016/j.oraloncology.2014.01.006

[48] BISHOP JA, GUO TW, SMITH DF, WANG H, OGAWA T et al. Human papillomavirus-related carcinomas of the sinonasal tract. Am J Surg Pathol 2013; 37: 185–192. http://dx.doi.org/10.1097/PAS.0b013e3182698673

[49] JIANG R, EKSHYYAN O, MOORE-MEDLIN T, RONG X, NATHAN S et al. Association between human papilloma virus/Epstein-Barr virus coinfection and oral carcinogenesis. J Oral Pathol Med 2015; 44: 28–36. http://dx.doi.org/10.1111/jop.12221

doi:10.4149/neo_2016_013

Supplementary Information

Human papillomavirus and Epstein-Barr virus in nasopharyngeal carcinoma in a non-endemic Eastern European population

M. SVAJDLER JR[1,2,3,*], J. KASPIRKOVA[1,2], R. MEZENCEV[4], J. LACO[5], T. TORDAY[6], P. DUBINSKY[7], L. STRAKA[8], O. ONDIC[1,2], M. MICHAL[1,2], A. SKALOVA[1,2]

[1]Sikl's Department of Pathology, Charles University in Prague, The Faculty of Medicine and Faculty Hospital in Pilsen, Czech Republic; [2]Biopticka laborator, s.r.o., Pilsen, Czech Republic; [3]Department of Pathology, Louis Pasteur University Hospital, Kosice, Slovakia; [4]Integrated Cancer Research Center, School of Biology and Parker H. Petit Institute of Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA, USA; [5]The Fingerland Department of Pathology, Charles University in Prague, The Faculty of Medicine and University Hospital in Hradec Kralove, Czech Republic; [6]Medicyt s.r.o., Kosice, Slovakia; [7]Department of Radiation Oncology, Oncology Institute, Kosice, Slovakia; [8]Klinicka patologia Presov, s.r.o., Presov, Slovakia

**Correspondence: svajdler@yahoo.com*

Methods

Meta-analysis-search strategy: Studies that reported HPV and EBV status of nasopharyngeal carcinoma specimens were identified by MEDLINE/PubMed search using various combinations of the key words "nasopharyngeal carcinoma", "human papillomavirus" and "Epstein-Barr virus without time limits. Additional relevant studies were identified among references cited in these retrieved reports. Articles that reported both EBV and HPV status of nasopharyngeal carcinoma (NPC) were reviewed and considered for meta-analysis (see Table 2 in the main text).

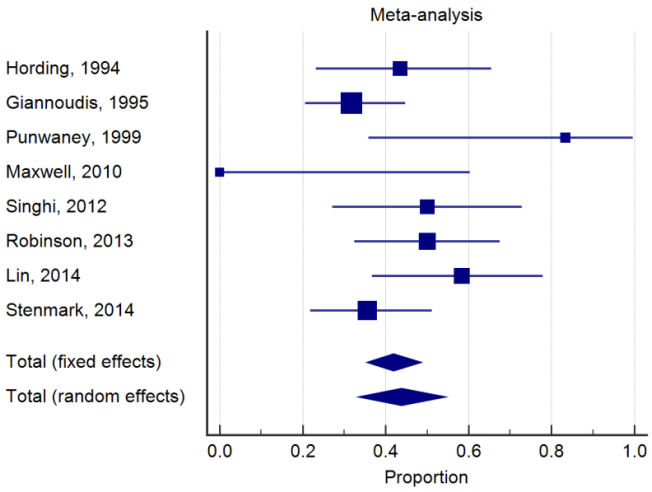
To estimate the prevalence of EPV-positive nasopahryngeal carcinoma in Caucasian patients, all studies that reported sample size and number of EBV- positive/negative cases detected by ISH or PCR among Caucasian patients were identified and included to meta-analysis.

Prevalence of HPV-positive NPC among Caucasian patients was estimated by separate meta-analysis for PCR-defined and ISH/p16-defined HPV positivity. Studies were included in these meta-analyses if they provided information on sample size and number of HPV positive/negative cases attributable to Caucasian patients by PCR or ISH/p16 IHC, respectively. Caucasian patients were defined as those having origins in any of the original people of Europe.

Meta-analysis-execution: Overall proportion of EBV- or HPV-positive cases was estimated by meta-analysis using the fixed effects and random effects models implemented in MedCalc for Windows, version 15.6 (MedCalc Software, Ostend, Belgium). The fixed effects model assumes that the studies share a common true effect that is estimated by the summary effect, while the random effects model assumes that true effects vary across studies and the summary effect is the weighted average of the reported in the different studies. Proportions reported by individual studies and estimated summary effects together with corresponding CI_{95} intervals were presented in forest plots (marker size is proportional to study weight). Consistency of proportions across individual studies included to meta-analyses was assessed by Cochran's Q and I^2 statistics. When heterogeneity was present, the random effects models were used for the interpretation of the results of meta-analysis.

Results

1 Meta-analysis of prevalence of EBV-positivity in NPC in Caucasian patients



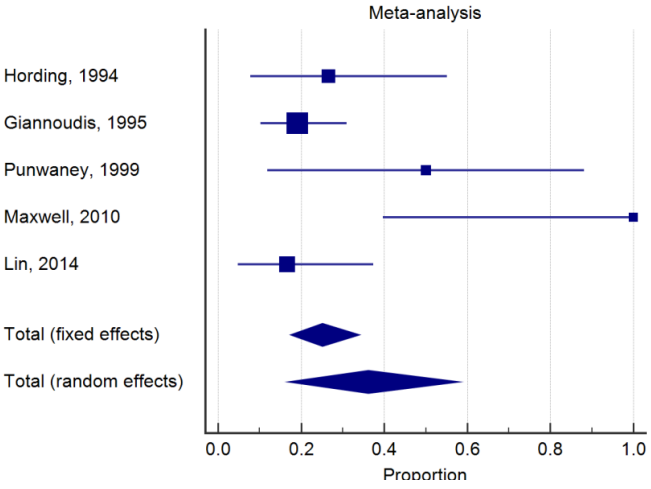
Study	Standard deviation	Proportion (%)	95% CI
Hording, 1994	23	43.5	23.2 to 65.5
Giannoudis, 1995	63	31.7	20.6 to 44.7
Punwaney, 1999	6	83.3	35.9 to 99.6
Maxwell, 2010	4	0.00	0.00 to 60.2
Singhi, 2012	20	50.0	27.2 to 72.8
Robinson, 2013	34	50.0	32.4 to 67.6
Lin, 2014	24	58.3	36.6 to 77.9
Stenmark, 2014	45	35.6	21.9 to 51.2
Total (fixed effects)	219	41.9	35.4 to 48.6
Total (random effects)	219	43.8	33.4 to 54.5

Test for heterogeneity

Q	16.2669
DF	7
Significance level	P = 0.0228
I^2 (inconsistency)	56.97%
95% CI for I^2	5.41 to 80.42

2 Meta-analysis of prevalence of HPV-positivity in NPC in Caucasian patients

2.1 Prevalence of HPV-positivity in NPC detected by PCR

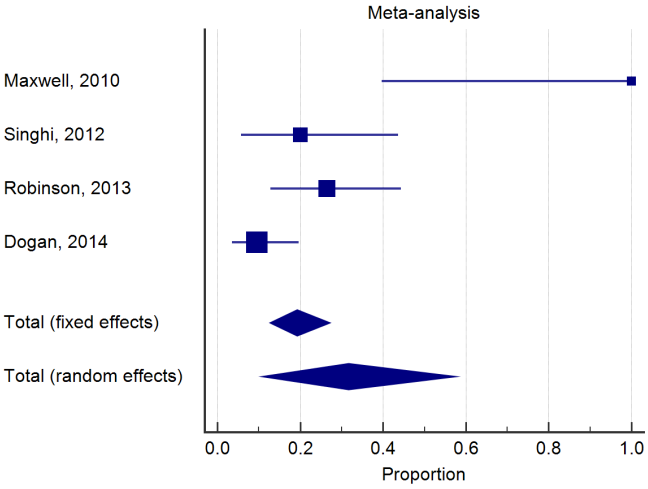


Study	Standard deviation	Proportion (%)	95% CI
Hording, 1994	15	26.7	7.8 to 55.1
Giannoudis, 1995	63	19.0	10.2 to 30.9
Punwaney, 1999	6	50.0	11.8 to 88.2
Maxwell, 2010	4	100.0	39.8 to 100.0
Lin, 2014	24	16.7	4.7 to 37.4
Total (fixed effects)	112	25.1	17.5 to 33.9
Total (random effects)	112	36.1	16.9 to 58.0

Test for heterogeneity

Q	17.1450
DF	4
Significance level	P = 0.0018
I^2 (inconsistency)	76.67%
95% CI for I^2	43.26 to 90.41

2.2 Prevalence of HPV-positivity in NPC detected by ISH/p16 IHC



Study	Standard deviation	Proportion (%)	95% CI
Maxwell, 2010	4	100.0	39.8 to 100.0
Singhi, 2012	20	20.0	5.73 to 43.7
Robinson, 2013	34	26.5	12.9 to 44.4
Dogan, 2014	63	9.52	3.58 to 19.6
Total (fixed effects)	121	19.2	12.7 to 27.2
Total (random effects)	121	31.7	10.7 to 57.6

Test for heterogeneity

Q	21.2752
DF	3
Significance level	P = 0.0001
I^2 (inconsistency)	85.90%
95% CI for I^2	65.47 to 94.24

References

HORDING U, NIELSEN HW, DAUGAARD S, ALBECK H. Human papillomavirus types 11 and 16 detected in nasopharyngeal carcinomas by the polymerase chain reaction. Laryngoscope 1994; 104: 99–102. http://dx.doi.org/10.1288/00005537-199401000-00018

GIANNOUDIS A, ERGAZAKI M, SEGAS J, GIOTAKIS J, ADAMOPOULOS G et al. Detection of Epstein-Barr virus and human papillomavirus in nasopharyngeal carcinoma by the polymerase chain reaction technique. Cancer Lett 1995; 89: 177–181. http://dx.doi.org/10.1016/0304-3835(94)03667-8

PUNWANEY R, BRANDWEIN MS, ZHANG DY, URKEN ML, CHENG R et al. Human papillomavirus may be common within nasopharyngeal carcinoma of Caucasian Americans: investigation of Epstein-Barr virus and human papillomavirus in eastern and western nasopharyngeal carcinoma using ligation-dependent polymerase chain reaction. Head Neck 1999; 21: 21–29. http://dx.doi.org/10.1002/(SICI)1097-0347-(199901)21:1<21::AID-HED3>3.0.CO;2-Z

MAXWELL JH, KUMAR B, FENG FY, MCHUGH JB, CORDELL KG et al. HPV-positive/p16-positive/EBV- negative nasopharyngeal carcinoma in white North Americans. Head Neck 2010; 32: 562–567.

SINGHI AD, CALIFANO J, WESTRA WH. High-risk human papillomavirus in nasopharyngeal carcinoma.Head Neck 2012; 34: 213–218. http://dx.doi.org/10.1002/hed.21714

ROBINSON M, SUH YE, PALERI V, DEVLIN D, AYAZ B et al. Oncogenic human papillomavirus-associated nasopharyngeal carcinoma: an observational study of correlation with ethnicity, histological subtype and outcome in a UK population. Infect Agent Cancer 2013; 8: 30. http://dx.doi.org/10.1186/1750-9378-8-30

LIN Z, KHONG B, KWOK S, CAO H, WEST RB et al. Human papillomavirus 16 detected in nasopharyngeal carcinomas in white Americans but not in endemic southern Chinese patients. Head Neck 2014; 36: 709–714. http://dx.doi.org/10.1002/hed.23362

DOGAN S, HEDBERG ML, FERRIS RL, RATH TJ, ASSAAD AM et al. Human papillomavirus and Epstein-Barr virus in nasopharyngeal carcinoma in a low-incidence population. Head Neck 2014; 36: 511–516. http://dx.doi.org/10.1002/hed.23318

STENMARK MH, MCHUGH JB, SCHIPPER M, WALLINE HM, KOMARCK C et al. Nonendemic HPV-Positive Nasopharyngeal Carcinoma: Association With Poor Prognosis. Int J Radiat Oncol Biol Phys 2014; 88: 580–588. http://dx.doi.org/10.1016/j.ijrobp.2013.11.246

Supplementary Table 1. Summary of the literature addressing both EBV and HPV virus status in nasopharyngeal carcinoma

Author, year [reference]	Number of NPC investigated (type of population)	Method of EBV/HPV detection	EBV positivity (%)	HPV positivity (%)	HPV type	Coinfection (%)	Comment
Tyan, 1993[7]	30 (Eastern Asian/ Taiwanese)	PCR/PCR	30/30 (100)	14/30 (47)	16	14/30 (47)	EBV and HPV present in 30/44 and 11/44 other H&N tumors
Hørding, 1994 [8]	38 (23 Danish, 15 Inuits)	PCR/PCR	10/23 (45) Dannish 15/15 (100) Inuits	4/15 (27) Dannish 0/15 (0) Inuits	16, 11	0 (0)	
Giannoudis, 1995 [9]	63 (Greek)	PCR/PCR	20/63 (32)	12/63 (19)	N/A	0 (0)	
Rassekh, 1998 [10]	17 (N/A)	PCR/PCR	15/17 (88.2)	9/17 (52.9)	16, 18, 33, 6, 7	9/17 (53)	Moderate keratinization present in 4/8 HPV-negative and in none of HPV-positive NPC
Punwaney, 1999 [11]	30 (6 Caucasian Americans, 1 Chinese American, 23 Korean and Chinese)	PCR/PCR	**10/13 (77) overall** 5/6 (83) Caucasian 5/7 (71) Asiatic	**7/30 (23) overall** 3/6 (50) Caucasian 4/24 (17) Asiatic	N/A	2/6 (33)	
Tung, 1999 [12]	88 (Eastern Asian/ Chinese)	PCR/PCR	73/88 (83)	45/88 (51)	16, 18	37/88 (42)	
Mirzamani, 2006 [13]	20 (Western Asian/ Iranian)	ISH/ISH	19/20 (95)	2/20 (10) HPV 6/11 2/20 (10) HPV 16/18	6/11, 16/18	3/20 (15)	
Maxwell, 2010 [14]	5 (4 White, 1 Asian)	ISH/PCR, p16 IHC	0/4 (0) White 1/1 (100) Asian	4/4 (100) White 0/1 (0) Asian	16, 18, 59	0 (0)	Good therapeutic response in HPV-positive NPC cases
Lo, 2010 [15]	30 (19 White + 5 Asian + 6 Other)	ISH/ ISH,PCR, p16 IHC	14/28 (50)	15/28 (54)	16	Yes, number N/A	4/26 HPV positive by p16 and ISH and PCR
Laantri, 2011 [16]	70 (North African/ Morrocan)	PCR/PCR	70/70 (100)	24/70 (34)	31, 16, 18, 33, 35, 45, 59	24/70 (34)	
Huang, 2011 [17]	43 (PCR group) + 46 (ISH group) (Eastern Asian/ Taiwanese)	ISH, PCR/ ISH, PCR	43/43 (100) PCR group 43/46 (94) ISH group	15/42 (35) PCR group 19/46 (41) ISH group	16, 18, 33, 58, 66, 69, 72, 84	15/43 (35) PCR group 18/46 (39) ISH group	Tumour high-risk HPV status did not correlate with the prognosis Oncogenic HPVs were not always retained in NPC cells during the process of metastasis (HPV present in 2/4 metastases) 14 /16 (88%) of the high-risk HPV-positive NPCs showed cytoplasmic/perinuclear (epigenetic) ISH staining pattern: study does not support an association between oncogenic HPV and the carcinogenesis
Singhi, 2012 [20]	45 (20 White, 11 Asian, 11 African American, 2 Middle Eastern, 1 Hispanic)	ISH/ISH, p16 IHC	**34/45 (76) overall** 10/20 (50) White 11/11 (100) Asian 10/11 (91) African American 2/2 (100) Middle Eastern 1/1 (100) Hispanic	**4/45 (9) overall** 4/20 (20) White	N/A	0 (0)	All 3 HPV positive NPC with staging information had extension into the oropharynx

Supplementary Table 1. Summary of the literature addressing both EBV and HPV virus status in nasopharyngeal carcinoma (continued)

Author, year [reference]	Number of NPC investigated (type of population)	Method of EBV/HPV detection	EBV positivity (%)	HPV positivity (%)	HPV type	Coinfection (%)	Comment
Robinson, 2013[21]	67 (34 White, 17 Asian, 14 Black, 2 North African)	ISH/ ISH, PCR, p16 IHC	**47/67 (70) overall** 17/34 (50) White 16/17 (94) Asian 12/14 (86) Black 2/2 (100) North African	**11/67 (16) overall** 9/34 (26) White 2/14 (14) Black	N/A	0 (0)	No statistically significant difference in overall survival outcome between patients with HPV-positive and HPV-negative NPC Radiology review performed
Lin, 2014 [22]	86 Asian/Chinese, 108 American (77 Asian, 25 White, 2 Black, 3 Hispanic, 1 Unknown)	ISH, PCR/ ISH, PCR, p16 IHC	**83/86 (97) Chinese** **93/104 (89) American** 74/74 (100) Asian 14/24 (58) White 2/2 (100) Black 3/3 (100) Hispanic 0/1 (0) Unknown	**0/86 (0) Chinese** **5/104 (5) American** 5/24 (21) White	N/A 16	0 (0)	None of HPV-positive NPC showed tumor involvement of the oropharynx White patients with EBV-negative NPC showed a trend toward worse overall survival
Dogan, 2014 [23]	90 (56 White, 4 Black, group 1981-2012; N/A for 1956-1971 group)	ISH/ISH, p16 IHC	53/92 (58)	6/63 (10) all White	N/A	0 (0)	None of HPV-positive NPC showed oropharyngeal or sinonasal involvement The overal surrvival of patients with HPV-positive NPC was not significantly different from that of EBV-positive NPC The overal surrvival of patients with EBV/HPV-negative NPC was worse
Stenmark, 2014 [24]	61 (45 White, 9 African American, 5 Asian, 2 Middle Eastern)	ISH/PCR, p16 IHC	**26/61 (43) overall** 16/45 (36) White 5/9 (56) African American 4/5 (80) Asian 1/2 (50) Middle Eastern	**18/61 (30) overall** 17/45 (38) White 1/9 (10) African American	16, 18, 59, 39, 45	0 (0)	HPVpositive and EBV/HPV-negative NPC were associated with worse outcomes Radiology review performed