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INVITED REVIEW

Human Papillomavirus in Non-Oropharyngeal Head and Neck Cancers: A Systematic Literature Review

Tatyana Isayeva · Yufeng Li · Daniel Maswahu · Margaret Brandwein-Gensler

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Abstract Perhaps one of the most important developments in head and neck oncology of the past decade is the demonstration that patients with human papillomavirus (HPV)-mediated oropharyngeal cancers have significantly improved outcomes, compared to HPV-negative counterpart patients. This has become the basis for clinical trials investigating the impact on "treatment deintensification" for patients with HPV-mediated oropharyngeal cancers. Unfortunately, the significance of HPV in non-oropharyngeal head and neck cancers is much less certain. Our goal is to systematically review the published data regarding the role HPV in carcinomas of the oral cavity, larynx, sinonasal tract and nasopharynx with respect to HPV detection frequency, viral activity, and association with outcome. We also present preliminary data on HPV16/18 transcriptional status in oral cavity carcinomas, as well as salivary gland neoplasia, as determined by nested reverse transcription PCR for HPV E6/E7 RNA. The *weighted prevalence* (WP) of HPV DNA detection in 4,195 oral cavity cancer patients is 20.2 %, (95 % CI 16.0 %, 25.2 %). HPV16 is the most common type detected. Importantly, no data currently demonstrates a significant association between the presence of HPV DNA and improved outcome. The WP of HPV DNA in 1,712 laryngeal cancer patients is 23.6 %, (95 % CI 18.7 %, 29.3 %). Similarly, no association has yet been demonstrated between HPV DNA status and outcome. The WP of HPV DNA detection in 120 sinonasal cancer patients is 29.6 % (95 % CI 17.8 %, 44.9 %), and in 154 nasopharyngeal carcinoma patients is 31.1 %, (95 % CI 20.3 %, 44.5 %). Recent preliminary data also suggests an association between HPV and certain salivary gland neoplasms. The clinical significance of these findings is unclear. The published data strongly support the need for studies on patients with oral and laryngeal carcinomas that will be powered to find any differences in clinical outcome with respect to HR-HPV and p16 overexpression.

Keywords HPV · Squamous carcinoma · Oral cavity · Laryngeal · Larynx · Mucoepidermoid carcinoma · Salivary

T. Isayeva · D. Maswahu
Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA

Y. Li · D. Maswahu
Department of Preventative Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

M. Brandwein-Gensler (✉)
Surgical Pathology Section, Division Anatomic Pathology, NP 3545, University of Alabama at Birmingham, 1802 6th Ave South, Birmingham, AL 35233-1932, USA
e-mail: MGensler@uab.edu

Introduction

Perhaps one of the most important developments in head and neck oncology of the past decade is the demonstration that patients with human papillomavirus (HPV)-mediated oropharyngeal cancers have significantly improved clinical outcomes, compared to patients with HPV-negative oropharyngeal carcinomas. Clinical trials are underway to investigate the impact of "treatment deintensification" for patients with HPV-mediated oropharyngeal cancers. The possibility of offering patients with HPV-mediated cancers less aggressive adjuvant therapy is especially relevant given the potential for devastating radiation toxicities. Can treatment deintensification strategies also be applied to HPV-positive cancers at non-oropharyngeal sites? Does the presence of HPV in non-oropharyngeal carcinomas represent viral-mediated carcinogenesis, or merely "bystander"

infection? Lastly, even if HPV promotes carcinogenesis in non-oropharyngeal head and neck cancers, does it impact clinical outcome? Compared to the state of knowledge regarding HPV and oropharyngeal cancers, the significance of HPV in non-oropharyngeal head and neck cancers is unfortunately uncertain. These questions are especially important as laryngeal and oral cavity cancers are more common than oropharyngeal cancers.

Most publications focus on HPV DNA detection frequencies in non-oropharyngeal head and neck cancers, and only a few studies have directly addressed the impact of HPV on clinical outcome. The goal of this article is to systematically review the published data regarding the role of HPV in carcinomas of the oral cavity, larynx, sinonasal tract, and nasopharynx with respect to detection frequency, viral activity, and association with outcome. We also present some emerging data on HPV in salivary neoplasia.

Methods

The Pubmed search engine was used to identify studies published in English published from January 2000 through March 2012 using the MeSH terms "HPV", "Human Papillomavirus", "Head and Neck Cancer", "Oral Cavity", "Laryngeal", "Larynx", "Sinonasal", "Nasal", "Nasopharynx", and "Polymerase Chain Reaction" (PCR). Oral cavity includes tongue (excluding tongue base), gum, floor of mouth, buccal mucosa, and hard palate. Data regarding the hypopharynx was not abstracted, for concern of possible anatomic overlap with oropharynx. The following arbitrary restraints were placed on this review; we excluded non-English manuscripts, manuscripts published before 2000, case reports, and investigations using non-PCR techniques or only in-situ PCR. If data were available using multiple techniques, only the PCR-generated data were extracted [1]. Only data generated from primary squamous cell carcinomas (SCC) were extracted, and we excluded reports if: (1) the detection methods were not well-detailed, and/or (2) HPV data could not be extracted per anatomic site. We took care to avoid tallying overlapping patient cohorts [2–13]. The final extracted data included: county, year of publication, anatomical site, HPV type, detection method of including primers, and summary of findings for cancers, patient controls, benign lesions, potentially premalignant lesions, and premalignant lesions, when available. For consistency, low-risk HPV data were grouped together with "HPV-positive, all types". The weighted prevalence (WP) with 95 % confidence intervals (CI) was calculated using Comprehensive Meta-Analysis, version 2 (Meta-Analysis.com). Weights are based on the inverse variance from the random effects analysis which includes the "within-studies" variance plus the "between-studies" variance.

Results

One hundred publications fulfilled the above criteria and serve as the basis of this review; [1–100] the great majority of them relied on HPV DNA detection by PCR analysis. Detection of HPV DNA supports the idea that HPV is "associated" with a cancer. However, it does not distinguish whether HPV is transcriptionally active and thus might promote carcinogenesis (e.g., "driver" infection) or transcriptionally inactive (referred to as "passenger" or "bystander" infection). The usual accepted criteria to support HPV-mediated carcinogenesis are: (1) demonstration of high-risk HPV E6/E7 RNA; (2) p16 overexpression; (3) viral integration; and lastly (4) wild type 53 protein. These criteria have important caveats. The p16 gene may be methylated in HPV-mediated cancers. HPV integration is not necessary for promoting carcinogenesis. Lastly, while the HR-HPV+/wild-type p53 profile is the expected genetic phenotype of never-smokers with HPV-mediated oropharyngeal cancers, this polarized relationship is not observed in patients with oral cavity cancer, as will be discussed [101]. Furthermore, nondisruptive p53 mutations can be demonstrated in HPV-mediated head and neck cancers, and are thought to have an additive impact on overall p53 functional loss [102].

HPV and Cancers of the Oral Cavity

Table 1 summarizes 60 publications on 4,195 patients with oral cavity SCC. A total of 705/4,195 oral cavity carcinomas contained HPV DNA, (all types); the WP is 20.2 %, (95 % CI 16.0 %, 25.2 %). No geographical differences were seen. A subgroup of 53 oral verrucous carcinomas were studied; 22/53 contained HPV DNA, with a higher WP, 47.2 % (95 % CI 13.6 %, 83.6 %), as compared to usual oral SCC [34, 37, 79]. The difference in the WP between oral verrucous carcinomas and usual-type SCC is not statistically significant ($p = 0.15$); this is probably due to the small number of oral verrucous carcinomas studied, and the wide 95 % confidence intervals.

The most common HPV type detected in oral cancers is HPV16. A notable outlier is a study from South Africa which detected only HPV18, and not HPV16, in patients with oral cancer [23]. HPV18 was detected in a smaller percentage of oral cancers, some oral carcinomas had dual infections with HPV16/18 [51, 70, 96]. Rarer types detected in oral cancers were HPV8, HPV31, HPV38, and HPV66 [56, 68, 93]. Low-risk HPV is rarely detected in oral cancers, and when present might represent a "bystander" infection rather than possibly "driver" infection [37, 42, 64, 68, 80].

Table 1 HPV DNA detection frequencies in oral cavity carcinomas

Author	Year	Country	Method, primers, amplicon detection	Number of HPV positive cancers	Total cancers studied	HPV positive cancers (%)
Badaracco	2007	Italy	PCR, MY09/MY11, GP5/GP6	8	60	13.3
Baez	2004	Puerto Rico	PCR, HPV16 E6/E7 ORF	13	36	36.1
Bagan	2007	Spain	PCR, MY09/MY11	0	6	0.0
Balderas - Laenza	2007	Mexico	PCR, MY09/MY11, GP5/GP6	26	62	41.9
Barwad	2011	India	PCR, MY09/MY11, not nested, agarose gel	16	34	47.1
Boscolo-Rizzo	2009	Italy	PCR, HPV16 specific primers	2	10	20.0
Bouda	2000	Greece	PCR	18	19	94.7
Boy	2006	South Africa	PCR, HPV16/18 specific primers	7	59	11.9
Braakhuis	2004	Netherlands	PCR, GP5/GP6, typing	6	106	5.7
Correnti	2004	Venezuela	PCR, MYO9/MY11, not nested, agarose gel, Digene Sharp Signal Assay typing	8	16	50.0
Dahlgren	2004	Scandinavia	PCR, GP5/GP6, CPI/CPIIG, agarose gel	2	85	2.4
Deng	2011	Japan	PCR, MYO9/MY11, GP5/GP6, E1 consensus primers	9	25	36.0
Dong	2003	USA	PCR, HPV16/18 specific primers	3	16	18.8
Elango	2011	India	PCR, MY09/MY11, GP5/GP6, HPV16 specific primers	30	60	50.0
El-Mofty	2003	USA	PCR, SPF10, INNO-LiPA line probe	0	15	0.0
Feher	2009	Hungary	PCR, MY09/MY11, GP5/GP6	31	65	47.7
Fischer	2003	Germany	PCR, L1 consensus primers	0	2	0.0
Fujita	2008	Japan	PCR, SPF10, sequencing	11	23	47.8
Furniss	2007	USA	PCR, SPF1A, SPF2B, HPV16 E6 specific primers	38	150	25.3
Gillison	2000	USA	PCR, MY09/MY11, HPV16E7 HPV18E7 Dot blot	10	84	11.9
Gonzalez	2007	Argentina	PCR, MY09/MY11, GP5/GP6,	15	25	60.0
Gudleviciene	2009	Lithuania	PCR, HPV16/18 specific primers, agarose gel	1	13	7.7
Ha	2002	USA	PCR, HPV16 E6/E7 primers, real time quantitative PCR	1	34	2.9
Halimi	2011	Iran	PCR, MY09/MY11 then typed, agarose gel	6	30	20.0
Hansson	2005	Scandinavia	PCR, MY09/MY11, GP5/GP6, agarose gel, sequenced	15	85	17.6
Harris	2011	USA	PCR, MY09/MY11, GP5/GP6, type specific primers	2	25	8.0
Herrero	2003	Multiple countries	PCR, GP5/GP6, enzyme immune assay typing	30	766	3.9
Ibieta	2005	Mexico	PCR, MY09/M11, GP5/GP6, typed	21	50	42.0
Jalouli	2010	India	PCR, MY09/M11, not nested, agarose gel, typed with HPV16/18 specific primers, and sequenced	15	62	24.2
Kaminagakura	2011	Brazil	PCR, GP5/GP6, agarose gel	22	114	19.3
Kansky	2006	Slovenia	PCR, MY09/M11, GP5/GP6, WD72, WD76, agarose gel, typing by restriction fragment length polymorphism	4	44	9.1
Klozar	2008	Czech	PCR, GP5/GP6, not nested, chemoluminescence detection of hybridized amplicon, sequencing	2	10	20.0
Klussmann	2001	Germany	PCR, consensus primers, HPV16 specific primers, real time PCR	4	22	18.2
Koppikar	2005	India	PCR, L1 primers and GP5/GP6	28	83	33.7
Koskinen	2003	Scandinavia	PCR, MY09/MY11,GP5/GP6, SPF10, INNO-LiPA typing, FAP 59/64, CP65/70, CP66/69, type specific real time PCR	7	13	53.8
Kristoffersen	2012	Scandinavia	PCR, MY09/MY1, GP5/GP6	8	50	16.0
Laco	2011	Czech Republic	PCR, GP5/GP6	3	24	12.5
Lopes	2011	England	PCR, GP5/6 Q-PCR HPV16/18	2	142	1.4

Table 1 continued

Author	Year	Country	Method, primers, amplicon detection	Number of HPV positive cancers	Total cancers studied	HPV positive cancers (%)
Luo	2007	Tapei	PCR, MY09/M11, GP5/GP6, typed by HPV gene chip	13	51	25.5
Montaldo	2010	Italy	PCR, MY09/M11, agarose gel, sequenced	21	68	30.9
Mork	2001	Scandinavia	PCR, GP5/GP6 CpI, CpII E1, E6 specific primers for HPV6/11/16/18/33	4	91	4.4
Neme	2006	Hungary	PCR, MY09/MY11, type specific, E2 for integration	33	79	41.8
Pannone	2012	Italy	PCR, MY09/M11, GP5/GP6, 8% polyacrylamide gel	3	6	50.0
Popovic	2010	Serbia	PCR, consensus primers typing	6	60	10.0
Ribeiro	2011	Multiple countries	PCR, MY09/MY11, no nesting, HPV16E7 specific primers, agarose gel, typing by restriction fragment length polymorphism	0	483	0.0
Ringstrom	2002	USA	PCR MY09/MY11, agarose gel, typing by restriction fragment length polymorphism	2	41	4.9
Ritchie	2003	USA	PCR, MY09/MY11 agarose gel, dot blot, then heminested PCR MY09. GP5	10	94	10.6
Saghravanian	2011	Iran	PCR, GP5/GP6	3	21	14.3
Sand	2000	Scandinavia	PCR, MY09/MY11, agarose gel	3	24	12.5
Schlecht	2011	USA	PCR, MY09/11, dot blot	5	38	13.2
Seraj	2011	Iran	PCR, HPV 16/18 specific primers, agarose gel	25	94	26.6
Sethi	2011	USA	PCR, SPF10, INNO-LiPA typing	33	120	27.5
Slebos	2006	USA	PCR, MY09/MY11, sequenced	0	15	0.0
Smeets	2007	Netherlands	PCR, GP5/GP6 real time quantitative PCR	9	30	30.0
Smith	2008	USA	PCR,MY09/MY11, GP5/GP6, then typed	27	166	16.3
Soderberg	2008	USA	PCR, MY09/MY11, GP5/GP6, then sequenced	1	18	5.6
Sugiyama	2007	Japan	PCR, HPV16 E7 specific primers, agarose gel	24	66	36.4
Tachezy	2005	Czech Republic	PCR, GP5/GP6, then sequenced	3	12	25.0
van Monsjou	2012	Netherlands	PCR, INNO-LiPA typing	2	20	10.0
Zhang	2004	China	PCR, HPV 16/18 E6 specific primers, agarose gel	54	73	74.0
Total				705	4,195	

HPV RNA and Oral Cavity Cancer

As mentioned, demonstrating viral oncogene transcription suggests, but does not prove, that viral-mediated carcinogenesis is mechanistically possible ("driver infection"). There is a paucity of published data regarding HR-HPV E6/E7 RNA in oral cancers [3, 7, 21, 81, 85]. The common approach of these studies is to perform reverse-transcription PCR on HPV-positive cancers. *Only four studies* demonstrated that HR-HPV E6/E7 RNA were present in a total of 17/20 (85 %) HPV-positive oral carcinomas tested [3, 7, 21, 85].

Another approach is to perform *parallel* PCR and reverse-transcription PCR assays for all specimens [81]. In a study of 109 patients with head and neck cancer from multiple anatomic sites, three oral cancers were DNA+/RNA− (reflecting either low-level or no transcription, and possibly "passenger" infections), three oral cancers were DNA+/RNA+ (possibly driver infections), and four other cancers were DNA−/RNA+ [81]. This last set of HPV transcriptionally-active, yet DNA-negative carcinomas is still consistent with the possibility of HPV-mediated carcinogenesis, and speaks to the idea of greater detection sensitivity of reverse-transcription PCR compared with PCR. We have studied a cohort of 89 consecutive patients with oral cavity SCC, and determined the rate of HPV16/18 E6/E7 RNA, by nested reverse transcription PCR on archival tumor samples (unpublished data). We demonstrated that 30 patients (33.7 %) had either HPV16 or HPV18; no double infections were present.

HPV, Oral Cavity Carcinoma, and Outcome

Only three published studies on patients with oral cavity carcinoma specifically examined the impact of HPV on outcome [51, 89, 101]. Kaminagakura studied 114 patients

and found a nonsignficant trend towards improved survival for 22 HPV-positive patients [51]. Sugiyama studied 66 patients in total, 62 with outcome data; 23 of these patients were HPV-positive [89]. They demonstrated a nonsignficant trend towards improved overall survival for HPV-positive oral cavity cancer patients [89]. Smith found no association with HPV and outcome for patients with oral carcinoma, based on either serology (116 patients, 13 with HPV positive serology) or tumor HPV detection (100 patients, 12 with tumors positive for HR-HPV DNA) [101]. Interestingly, in our unpublished cohort of 89 patients with oral cavity carcinoma, no significant association was found for patients with either HPV16/18 E6E7 RNA and time to disease progression or disease specific survival.

Oral HPV in Control Populations

Table 2 addresses the issue of HPV oral reservoirs in healthy controls populations: there are 22 studies on 5,095 healthy patients. Most studies examined shed cells and/or saliva, which cannot distinguish between oral cavity and oropharyngeal viral reservoirs; both Klussmann [55] and Anderson [15] examined tonsillectomy specimens. However, all of these studies address the issue of intraoral HPV prevalence in healthy populations. A total of 259/5,095 normal controls had detectable HPV DNA (all types); the WP was 6.9 %, (95 % CI 3.5 %, 13.2 %). Interestingly, Smith and colleagues demonstrated a bimodal age distribution with the two prevalence peaks of 2.5 % for children under 1 year, and 3.3 % for volunteers between ages 16 and 20 [103]. Some of the relatively larger studies from India [30], Scandinavia [60], and China [96] demonstrate significantly greater oral HPV carrier rates as compared to the other studies, suggesting that real geographic differences may be present.

The WP of intraoral HPV detection is significantly lower than the rate of HPV detection in oral carcinomas (6.9 vs. 20.2 %, respectively, $p = 0.0002$). The findings suggest that HPV may contribute to oral cancer carcinogenesis. However, this relationship does not establish causality.

HPV in Benign, Potentially Premalignant, and Premalignant Oral Lesions

Table 3 addresses the issue of HPV detection frequency in a spectrum of oral lesions, summarizing cross-sectional data for DNA detection (all HPV types) in patients with

Table 2 HPV DNA detection frequencies in oral/oropharyngeal controls from healthy patients

Author	Year	Country	Number of positive normal specimens	Total specimens studied	HPV positive oral/oropharyngeal controls (%)
Anderson	2007	Scotland	0	24	0.0
Deng	2011	Japan	1	47	2.1
D'Souza	2007	USA	11	200	5.5
Elango	2011	India	31	46	67.4
Feher	2009	Hungary	3	72	4.2
Fujita	2008	Japan	7	10	70.0
Gonzalez	2007	Argentina	0	60	0.0
Hansson	2005	Scandinavia	15	320	4.7
Herrero	2003	Multiple countries	91	1,527	6.0
Jimenez	2001	Venezuela	2	20	10.0
Kansky	2006	Slovenia	3	45	6.7
Klussmann	2001	Germany	0	14	0.0
Koppikar	2005	India	5	102	4.9
Kristoffersen	2012	Scandinavia	28	50	56.0
Luo	2007	Tapei	8	90	8.9
Migaldi	2012	Italy	1	81	1.2
Montaldo	2010	Italy	0	52	0.0
Pannone	2012	Italy	0	15	0.0
Pinto	2008	Canada	6	129	4.7
Ribeiro	2011	Multiple countries	2	898	0.2
Saghravanian	2011	Iran	0	18	0.0
Smith	2007	USA	23	1235	1.9
Zhang	2004	China	22	40	55.0
Total			259	5,095	

benign lesions, potentially premalignant lesions, (leukoplakia, lichen planus, submucosal fibrosis), and premalignant lesions (high-grade dysplasia). Only two studies addressed *benign* oral lesions: HPV was detected in 23/48 (47.9 %) of benign biopsies. No conclusions or comparisons can be drawn due to the limited nature of this data.

Ten reports studied a group of lesions classified as "potentially premalignant" oral lesions (leukoplakia, lichen planus, submucous fibrosis); HPV DNA was detected in 54/144 lesions; the WP is 41.4 %, (95 % CI 25.8 %, 58.9 %), which is also significantly higher than the intraoral HPV carrier rate. This also suggests that HPV may also promote potentially premalignant lesions, although it does not establish causality. Finally, only two studies investigated premalignant oral lesions (high-grade dysplasia): HPV was detected in 6/63 (9.5 %) of cases. No conclusions can be made due to the paucity of data.

Interestingly, one small study demonstrated that patients with submucous fibrosis demonstrated the highest HPV prevalence (11/12, or 91.7 %) [49]. Oral submucous fibrosis is a potentially premalignant condition caused by areca nut chewing and histologically characterized by increased submucosal collagen deposition and squamous mucosal atrophy. Access to basal reserve cells is required for establishing HPV mucosal infection. The transitional zone between uterine ectocervix and endocervix is an example of a region particularly vulnerable to HPV infection. It is possible that oral mucosal atrophy due to submucous fibrosis allows for greater exposure of epithelial basal cells, and therefore greater vulnerability to HPV infection. There is a need to follow-up with larger studies investigating the incidence of HPV in patients with betel nut-induced submucous fibrosis.

HPV and Cancers of the Larynx

Disproportionately fewer laryngeal cancers have been studied for HPV as compared to oral cancers (1,712 vs. 4,195, respectively) within the same time period (2000–2012) despite the fact that global incidences for these two cancers are on the same order of magnitude [104]. Table 4 summarizes the 41 publications on 1,712 patients with laryngeal SCC. HPV DNA has been detected in 436/1,712 laryngeal cancers; the WP is 23.6 %, (95 % CI 18.7 %, 29.3 %). No geographical differences were seen. Only three laryngeal verrucous carcinomas were studied, HPV was detected in 2/3 tumors, one was low-risk (LR) and the other was high-risk (HR) [39].

The most common HPV type detected in laryngeal cancers is HPV16. Compared to oral carcinomas, a greater diversity of other HPV types has been detected: HPV18, HPV26, HPV31, HPV33, HPV39, HPV36, HPV45, HPV51, HPV52, HPV58, HPV59, HPV66, and HPV69 [8, 9, 14, 20, 26, 29, 39, 65, 71, 90, 94]. Low-risk HPV are uncommonly detected, and might represent incidental "bystander" rather than possibly "driver" infection [29, 39, 65, 75, 90, 94]. Rarely, integrated low-risk HPV has been found; viral integration does suggest viral-mediated carcinogenesis [90, 94].

Table 3 HPV DNA detection frequencies in benign, potentially premalignant (PPM), and premalignant (PM) oral specimens

Author	Year	Country	Number HPV positive benign Bx	Total benign Bx studied	HPV positive benign (%)	Number PPM HPV positive Bx	Total PPM Bx	HPV positive PPM (%)	Number PM HPV positive Bx	Total PM Bx studied	HPV positive PM (%)
Bagan	2007	Spain				0	4	0			
Bouda	2000	Greece				25	29	86.2	5	5	100
Feher	2009	Hungary				57	163	35.0			
Gonzalez	2007	Argentina	1	8	12.5	15	31	48.4			
Ha	2002	USA				0	44	0	1	58	1.7
Jalouli	2010	India				11	12	91.7			
Jimenez	2001	Venezuela	22	40	55						
Kristoffersen	2012	Scandinavia				32	50	64			
Luo	2007	Tapei				14	46	30.4			
Saghravanian	2011	Iran				0	19	0			
Sand	2000	Scandinavia				8	29	27.6			
Total			23	48		54	144		6	63	

Bx Biopsy

Potentially premalignant = Leukoplakias, Lichen Planus, Oral Submucousal Fibrosis [49]

Premalignant = High-grade dysplasias

Table 4 HPV DNA detection frequencies in laryngeal carcinomas

Author	Year	Country	Method, primers, amplicon detection	Number of cancers HPV+	Total cancers studied	Cancers HPV+ (%)
Almadori	2001	Italy	PCR, MY09/MY11, enzyme immune assay typing	15	42	35.7
Anderson	2007	Scotland	PCR, GP5/GP6, real time quantitative PCR	2	64	3.1
Badaracco	2007	Italy	PCR, MY09/MY11, GP5/GP6	4	30	13.3
Baez	2004	Puerto Rico	PCR, HPV16E6/E7 ORF	24	52	46.2
Baumann	2009	USA	PCR, GP5/GP6, enzyme immune assay typing	6	38	15.8
Boscolo-Rizzo	2009	Italy	PCR, HPV16 specific primers	1	38	2.6
Deng	2011	Japan	PCR, MY09/MY11, GP5/GP6, E1 consensus primers	2	16	12.5
Duray	2011	Belgium	PCR, GP5/GP6, type specific primers and real time quantitative PCR	44	59	74.6
El-Mofty	2003	USA	PCR, SPF10, INNO-LiPA line probe	2	7	28.6
Fakhry	2008	USA	PCR, MY09/MY11, Roche Molecular systems probe array	0	34	0.0
Fischer	2003	Germany	PCR, L1 consensus primers	13	34	38.2
Furniss	2007	USA	PCR, SPF1A, SPF2B, HPV16E6 specific primers	14	45	31.1
Gillison	2000	USA	PCR, MY09/MY11, HPV16/18E7 specific primers	16	86	18.6
Gudleviciene	2009	Lithuania	PCR, HPV16/18 specific primers, gel	6	18	33.3
Guvenc	2008	Turkey	PCR, nested MY09/MY11, GP5/GP6	7	50	14.0
Hassumi	2012	Brazil	PCR, GP5/GP6	7	53	13.2
Kleist	2004	Germany	PCR, MY09/MY11, types specific primers, polyacrylamide gels, sequencing	6	38	15.8
Klussmann	2001	Germany	PCR, consensus primers, HPV16 specific primers	1	14	7.1
Koppikar	2005	India	PCR, probably MY09/MY11	0	2	0.0
Koskinen	2007	Scandinavia	PCR, MY09/MY11, GP5/GP6, SPF10, INNO-LiPA line probe	3	69	4.3
Liu	2010	China	PCR, GP5/6, HPV16/18 specific primers, agarose gel	29	84	34.5
Major	2005	Hungary	PCR, MY09/MY11, GP5/GP6, HPV 6/11/16 type specific primers, agarose gel	8	16	50.0
Manjarrez	2006	Mexico	PCR, L1C1/L1C2, typing by restriction fragment length polymorphism	2	16	12.5
Mork	2001	Scandinavia	PCR, GP5/GP6, CpI, CpII, HPV16 type specific primers	1	32	3.1
Morshed	2010	Poland	PCR, SPF10, agarose gel, enzyme immune assay typing, INNO-LiPA genotyping	33	93	35.5
Oliveira	2006	Brazil	PCR, GP5/GP6, HPV type specific primers	41	110	37.3
Reidy	2004	USA	PCR, HPV type specific primers, agarose gel	6	6	100.0
Ringstrom	2002	USA	PCR, MY09/MY11, agarose gel, typing by restriction fragment length polymorphism	1	10	10.0
Schlecht	2011	USA	PCR, MY09/11, dot blot	8	32	25.0
Sethi	2011	USA	PCR, SPF10, INNO-LiPA line probe	26	111	23.4
Slebos	2006	USA	PCR, MY09/MY11, sequenced	1	9	11.1
Smith	2008	USA	PCR, MY09/MY11	4	40	10.0
Smith	2000	USA	PCR, MY09/MY11, agarose gel, sequenced	11	44	25
Snietura	2011	Poland	PCR (Abbott Molecular Real Time High-Risk HPV)	0	65	0.0
Stephen	2012	USA	PCR, HPVE6 specific primers, real time quantitative PCR	21	77	27
Szladek	2005	Hungary	PCR, MY09/MY11, GP5/GP6, then typed	12	25	48.0
Torrente	2005	Chile	PCR, MY09/MY11, E2 for integration, typing by restriction fragment length polymorphism	10	31	32.3
Van Houten	2001	Netherlands	PCR, GP5/GP6, enzyme immune assay typing	0	5	0.0

Table 4 continued

Author	Year	Country	Method, primers, amplicon detection	Number of cancers HPV+	Total cancers studied	Cancers HPV+ (%)
Van Monsjou	2012	Netherlands	PCR, INNO-LiPA line probe	0	2	0.0
Venuti	2000	Italy	PCR, MY09/MY11, E2 for integration, typing by restriction fragment length polymorphism	13	25	52.0
Vlachtsis	2005	Greece	PCR, "consensus primers"	36	90	40.0
				436	1,712	

HPV RNA and Laryngeal Cancer

HPV RNA was studied in only four publications and was detected in 8 of 10 HPV positive laryngeal carcinomas tested [2, 3, 21, 81].

HPV, Laryngeal Carcinoma, and Outcome

Only four published studies, Duray [29], Morshed [8, 9], Stephen [88], and Vlachtsis [95] examined the impact of HPV on the outcome of a total of 319 patients; 134 were HPV positive. No association of HPV status with outcome was found.

Laryngeal HPV in Control Populations

Table 5 addresses the issue of latent laryngeal HPV infection in control populations (usually autopsies or laryngeal brushings), and summarizes DNA detection data for five studies, all HPV types, in normal larynges. HPV DNA has been detected in 12/107 normal larynges; the WP is 9.6 %, (95 % CI 2.9 %, 27.2 %). There is a nonsignficant trend comparing the WP for laryngeal "HPV carrier rate" and HPV detection rate in laryngeal carcinomas, $p = 0.11$. This suggests that HPV might promote laryngeal cancer, but does not establish causality.

HPV in Other Laryngeal Lesions

The association of HPV6/11 with laryngeal papillomas is well-established [105]. On the other hand, very few studies have examined the rate of HPV detection in other *benign* laryngeal lesions. Morshed [8] did not find any HPV in 22 vocal cord nodules. Duray [29] studied 35 biopsies of vocal nodules (n = 20), chronic laryngitis (n = 13) and papillomas (n = 6) and detected HPV in 27/35 (77 %) of these specimens; however, the HPV detection rate was not subclassified by histology. Smith [10] detected HPV in 3/10 (30 %) laryngeal leukoplakias. Lastly, Morshed [8] detected HPV in laryngeal mucosa adjacent to cancer in 4/49 (8.2 %) cases. No comparisons or conclusions can be drawn from this limited data.

Table 5 HPV DNA detection frequencies in normal larynges

Author	Year	Country	Number HPV positive normal	Total normal studied	HPV positive laryngeal normal (%)
Guvenc	2008	Turkey	0	50	0.0
Kleist	2004	Germany	0	5	0.0
Smith	2000	USA	2	12	16.7
Szladek	2005	Hungary	10	40	25.0
Total			12	107	

HPV in Sinonasal Cancers

The association of HPV with Schneiderian inverted papillomas (IP) is well-established; the HPV WP is 25.2 % (95 % CI 14.7, 35.6 %), for IP studied by PCR with consensus primers [106]. The HPV WP significantly increases for IP with high-grade dysplasia (WP 55.8 %, 95 %CI 30.5, 81.0 %) and IP with carcinoma (WP 55.1 %, 95 %CI 37.0, 73.2 %), as compared to combined IP without dysplasia plus with mild-dysplasia (WP 22.3 %, 95 %CI 15.9, 28.6 %) ($p < 0.02$, Wald t test). The published findings support the role of low-risk HPV in the etiology of benign Schneiderian IP, and the idea that high-risk HPV is responsible for malignant progression of IP [106].

The published data specifically regarding sinonasal SCC and HPV are quite sparse and *suffers from heterogeneity with respect to association with IP*. Table 6 summarizes 9 publications of 120 patients with sinonasal carcinoma: HPV DNA (all types) was detected in 31/120 cancers and the WP is 29.6 % (95 % CI 17.8 %, 44.9 %). HPV16 is most commonly detected. Because of the heterogeneity of this group, no conclusions can be drawn from this WP.

HPV RNA and Sinonasal SCC

Only one publication looked at HPV transcriptional activity; two HPV+ SCC arising ex-inverted papillomas also reveal HPV RNA transcription, and evidence of viral integration [66].

Table 6 Frequencies of HPV DNA detection in sinonasal carcinomas

Author	Year	Country	Method, primers, amplicon detection	Number HPV positive cancers	Total cancers studied	HPV positive cancers (%)
Alos	2009	Spain	PCR, SPF10, INNO-LiPA typing	12	60	20.0
McKay	2005	USA	PCR, MY09/MY11, agarose gel	2	3	66.7
Kraft	2001	Switzerland	PCR, MY09/MY11	0	4	0.0
Hoffman	2006	Germany	PCR, MY09/MY11, HPV6/11/16 specific primers	4	20	20.0
Mork	2001	Scandinavia	PCR, GP5/GP6, CpI, CpII, HPV16 specific primers	0	4	0.0
Deng	2011	Japan	PCR, MYO9/MY11, GP5/GP6, E1 consensus primers	3	10	30.0
Badaracco	2007	Italy	PCR, MY09/MY11,GP5/GP6	2	2	100.0
Fischer	2003	Germany	PCR, L1 consensus primers	4	4	100.0
Sethi	2011	USA	PCR, SPF10, INNO-LiPA typing	4	13	30.8
Total				31	120	

HPV, Sinonasal SCC, and Outcome

Only one publication specifically examined clinical outcome for patients with sinonasal carcinomas, with respect to HPV status. Alos and colleagues studied 60 patients with sinonasal SCC, 12 arose ex-IP. Twelve SCC were HPV-positive, including one SCC-ex-IP. Patients with HPV+ sinonasal SCC had significantly improved 5-year progression-free survival rates, 62 % (95 % CI 23 %, 86 %) versus 20 %, (95 % CI 9 %, 34 %, $p = 0.004$ log rank test), and overall survival rates, 80 % (95 % CI 20 %, 95 %) versus 31 % (95 % CI 14 %, 47 %, $p = 0.036$ log rank test) [97].

There are limited data regarding sinonasal HPV "carrier" rates. Hoffmann [46] found HPV in 1 of 39 (2.6 %) sinonasal polyps.

HPV in Nasopharyngeal Carcinoma (NPC)

There has been recent interest in the role of HPV in NPC. Table 7 summarizes the 8 PCR-based studies that include 154 patients with NPC. Six of these studies have been published within the last 3 years. HPV 16 was detected. An important caveat to consider is that carcinomas may be of oropharyngeal origin with extension into the contiguous nasopharynx, rather than represent primary NPC. Having said this, three recent studies examined the issue of HPV/Epstein Barr virus (EBV) co-infection by various techniques [98, 99, 107]. These studies suggest a dichotomy of HPV+/EBV− NPC (14/68 cases, 20.5 %) versus HPV−/EBV+ NPC (40/80, 50 %) with no tumors harbouring double HPV/EBV infections [98, 99, 107]. However, NPC with double HPV/EBV infections have been reported [108].

HPV RNA in NPC

HPV RNA has been detected in the single HPV+ NPC studied [81].

HPV, NPC, and Outcome

Only one publication specified the outcomes for five patients with NPC, four of whom were HPV+/EBV−, and the remaining patient was HPV−/EBV+ [98]. The limited nature of this data precludes further discussion.

HPV DNA in Salivary Neoplasia

The SEER 9 data demonstrates a trend of increasing incidence for mucoepidermoid carcinoma (MEC) in women, ages 15–34 years [109] reminiscent of the significantly increased incidence of oropharyngeal cancers over the past three decades due to HR-HPV-mediated carcinogenesis. This raises the interesting question as to whether HR-HPV can also be involved in MEC carcinogenesis [109]. The possibility of HPV promoting salivary tumors has been addressed in the literature in a limited manner. Vageli demonstrated HPV16/18 DNA in seven of nine parotid tumors, including an oncocytoma, acinic cell carcinoma, Warthin's tumor, and a pleomorphic adenoma [110]. While DNA detection does not address the issue of transcriptional activity, and therefore biological relevance, these authors demonstrated relatively high copy number by quantitative real-time PCR for some tumors, which suggests a causative relationship. Recently, Boland and colleagues demonstrated HR-HPV DNA in two of 16 salivary adenoid cystic carcinomas using the Ventana in situ hybridization (ISH) probes [111]. HPV DNA ISH is not the optimum technique

Table 7 HPV DNA detection frequencies in nasopharyngeal carcinoma

Author	Year	Country	Method, primers, amplicon detection	Number HPV positive cancers	Total cancers studied	HPV positive cancers (%)
Barwad	2011	India	PCR, MY09/MY11, not nested, agarose gel	1	20	5.0
Deng	2011	Japan	PCR, MY09/MY11, GP5/GP6, E1 primers	3	9	33.3
Klussmann	2001	Germany	PCR consensus primers, HPV16 specific primers	1	13	7.7
Lantri	2011	Morrocco	PCR, MY09/MY11, nitrocellulose gel with ISH using type specific probes	24	70	34.3
Lo	2010	USA	PCR with type-specific E6 primers to HPV16/18, agarose gel	12	28	42.8
Maxwell	2009	USA	PCR, multiplex competitive PCR with type-specific E6 primers to multiple HR HPV	4	5	80
Mork	2001	Scandinavia	PCR, GP5/GP6, CpI, CpII E1 consensus primers, HPV16 specific primers	1	7	14.3
Schlecht	2011	USA	PCR, MY09/11 dot blot	1	2	50
Total				47	154	

for initial exploratory studies. The detection sensitivity of ISH might be very good depending on the context. However, greater tumor sampling is accomplished by PCR on formalin-fixed, paraffin-embedded (FFPE) samples, as compared to ISH, and is therefore the preferred approach for initial exploratory studies.

HPV RNA in Salivary Neoplasia

We studied a cohort of 89 patients with MEC for high risk HPV E6/E7 RNA by nested reverse transcription PCR (unpublished data). A total of 42 patients (47.2 %) had either HPV 16 or 18, and seven (7.1 %) had both 16 and 18. Interestingly, there was no predilection of HPV positivity in minor salivary MEC as compared to MEC of the major glands. Eighty four cases were studied by IF with the monoclonal C1P5 antibody which detects E6 protein of both HPV16/18. Eighteen tumors displayed nuclear and or cytoplasmic tumor staining, and the protein was detected in both mucinous and squamoid elements (Fig. 1). All cases positive by IF were HPV16/18 positive by RT-PCR. Fourteen additional MEC were negative by IF and positive by RT-PCR. This preliminary data demonstrates that transcriptionally active HPV16/18 is common in MEC. Thus, HPV may possibly promote the carcinogenesis of these tumors.

The significance of the HPV DNA and RNA in salivary neoplasia is presently unknown.

Discussion

Technical Considerations

Despite limiting this systematic literature review to PCR-based studies, the heterogeneity of published data begs the issue of technical considerations, and should be briefly addressed. The gold standard for HPV assay sensitivity is the detection of HPV RNA in frozen tissues [81, 85]. HPV RNA can be present in excess in HPV DNA, and snap frozen tissue does not undergo the extensive nucleic acid/protein cross-linking and continuous DNA/RNA degradation found in FFPE samples. Most, but not all cited studies used FFPE samples. Tables 1 and 4 demonstrate that nested PCR using the MY09/MY11 consensus primers, and nested GP5+/GP6+ consensus primers, is a very common approach. These consensus primers detect a *broad spectrum of low-risk and high-risk mucosal HPV types* [112]. However, there are two major limitations with this approach: (1) size of the region to be amplified; and (2) potential loss of the L1 gene.

The MY09/MY11 primers amplify a region 450 base pairs long, which is too long for amplifying FFPE samples. The GP5+/GP6+ consensus primers detect a much smaller region, on the order of 150 bp. In general, a nested approach increases amplicon concentration, allowing for better amplicon visualization. *However, nested PCR does not abrogate the initial sensitivity bottleneck of using the MY09/MY11 consensus primers*. We recommend a nested approach using the GP5 +/GP6 + consensus primers for both rounds of PCR. The SPF10 (Short PCR Fragment) primers amplifies a region in the L1 gene only 65 bp long, so may be even more sensitive than the GP5+/GP6+ primers; however, we have not had direct experience with these primers.

The second limitation is that these primers detect sequences in the L1 region which can be lost upon HPV integration. If HPV is present in both integrated and episomal forms, one would expect positive results. However, if HPV is entirely integrated, PCR using these consensus primers may result in a false negative reaction. This was

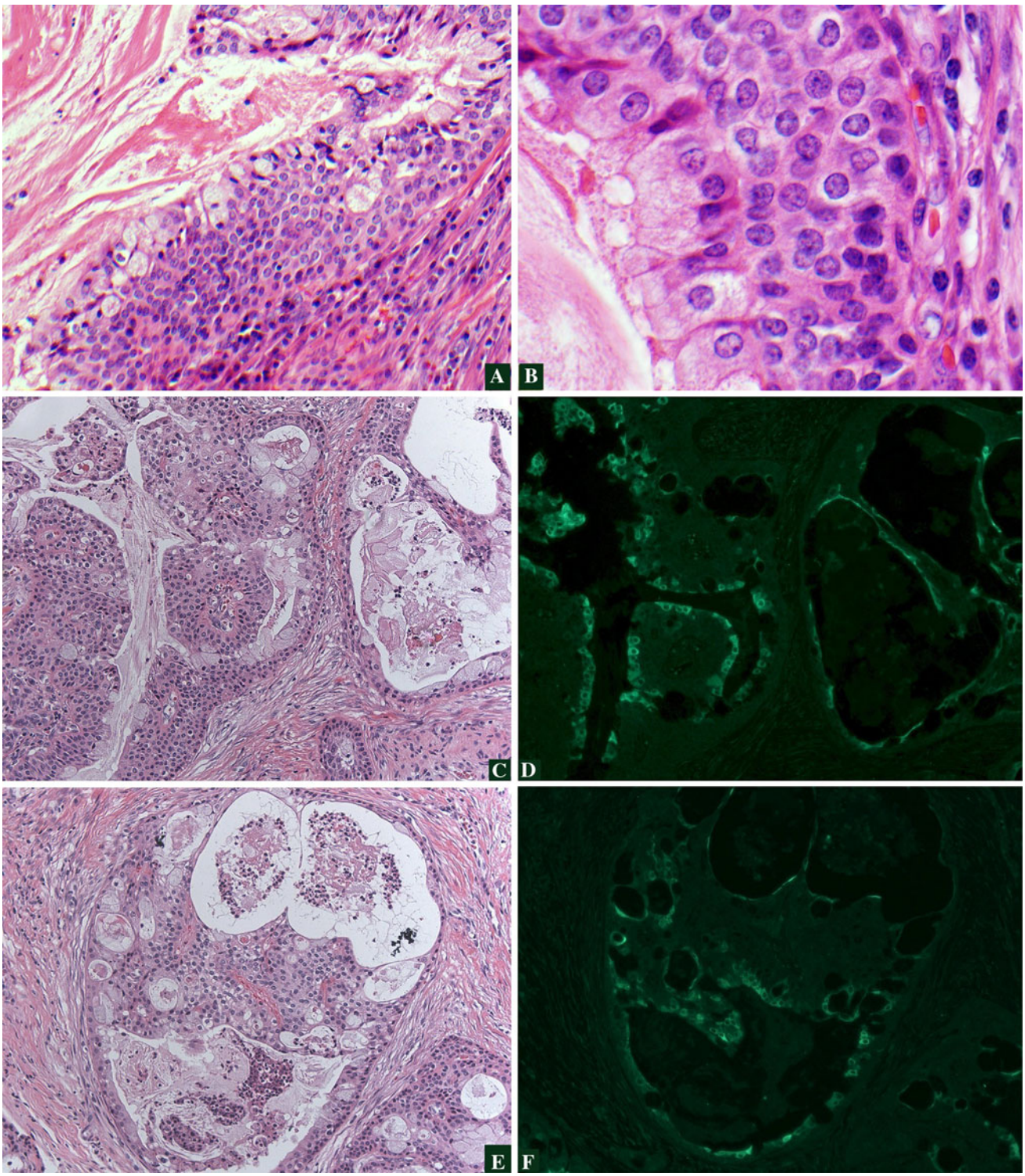

Fig. 1 *HPV and Histology* **a**, **b** demonstrate low- and high-power magnification, respectively, of an HPV-positive, cystic, low-grade MEC with proliferation of basaloid type cells. *Immunofluorescence (IF) for HPV16/18 E6 protein in MEC* **c**, **e** demonstrate hematoxylin and eosin stained areas of a MEC which is HPV-positive. **d**, **f** represent the corresponding regions demonstrating positive IF staining using an antibody to HPV16/18 E6 protein. Tumor nuclear and cytoplasmic staining is seen (*bright green*) which correlates with both the glandular and squamoid elements

nicely demonstrated by Duray [29] who detected HPV in an additional 36 % of laryngeal SCC when assayed using type-specific E6/E7 primers. These laryngeal carcinomas harbored HPV that was entirely integrated and resulted in false negative PCR with GP5+/GP6+ primers, but positive PCR with E6/E7 type-specific primers. One can then use the CPIIG/CPI primer pair, which amplifies a 188-bp fragment in the highly conserved E1 ORF region of various skin and mucosal HPV, for specimens negative by PCR with GP5+/GP6+ primers.

General Conclusions

This systematic literature review highlights the present state of our knowledge with respect to three fundamental questions: (1) Is HPV associated with cancers of oral cavity, larynx, sinonasal tract, nasopharynx, and salivary glands? (2) Is there a causative relationship? (3) If HPV mediates carcinogenesis in these sites, does this impart an improved survival akin to HPV-mediated oropharyngeal SCC? As mentioned, this is important as an improved survival could be exploited to develop new treatment strategies. Each non-oropharyngeal site will be summarized with respect to these questions.

HPV and Oral Cavity Cancer

Our systematic review, plus other published reviews support the idea that HPV is significantly present in a subgroup of oral cavity SCC, as compared to control populations; thus, HPV may possibly contribute to oral carcinogenesis. We find that the WP for HPV DNA detection in 60 studies on 4,195 patients is 23.3 %, (95 % CI 18.1 %, 28.5 %). Kreimer reviewed 35 PCR-based studies on 2,642 patients with oral cavity SCC and determined the *cumulative pooled prevalence* for HPV DNA and oral SCC to be 23.5 %, (95 % CI 21.9, 25.1) [113]. Termine reviewed 47 PCR studies on 4852 patients with oral cavity SCC and determined the *cumulative pooled prevalence* of HPV DNA to be 39.9 %, (95 % CI 30.2, 49.8) [114]. A limitation of cumulative pooled prevalence is that it assumes homogeneity among the pooled samples. The WP adjusts for standard error per study, and between studies, minimizing the variability of pooled estimates.

Another approach is to compare the odds ratio (OR) for the association of HPV in controls versus cancer patients. Syrjänen reviewed 39 studies of 1,885 patients with oral SCC and 2,248 controls, and demonstrated an OR of 3.98 (95 % CI 2.6, 6.0) for HPV (all types), and OR of 3.86 (95 % CI 2.2, 6.9) for HPV16 [115].

There is limited published data to support causation in this context. Only four studies demonstrated HR-HPV E6/E7 RNA to be present in a total of 17/20 (85 %) HPV positive oral carcinomas tested [3, 7, 21, 85]. We also mentioned our unpublished findings of HPV16/18 E6/E7 RNA in 33.7 % of 89 oral cavity SCC studied by nested reverse transcription PCR. Future studies should address the issue of HPV RNA in oral cavity SCC.

P16 expression status should also be studied specifically in this context of oral cavity SCC, with the important caveat that lack of p16 overexpression does not exclude the possibility of HPV-potentiated carcinogenesis. Most publications correlating p16 expression with HPV status study mixed groups of cancers from different anatomic sites [61, 81, 93]. These studies are not powered to determine the sensitivity and specificity of p16 overexpression as a surrogate biomarker for HPV-mediated oral cavity carcinogenesis. Importantly, future studies should utilize whole tissue section staining rather than tissue microarrays, and specifically document the intensity and distribution of p16 overexpression in oral cavity SCC to access test performance at various "cut-points".

With respect to etiology, the interaction between HPV, cigarettes, and alcohol exposure is more complex in the oral cavity as compared to the oropharynx. Smith recently reported that among heavy tobacco users, the risk of oropharyngeal carcinoma is greater in HPV-seronegative patients (adjusted OR = 11.0) compared with HPV-seropositive patients (adjusted OR = 4.7); among heavy alcohol users the risk is also greater in HPV-seronegative patients (adjusted OR = 24.3) compared to HPV-seropositive patients (adjusted OR = 8.5) [101]. This is consistent with the concept of mutually exclusive pathways of HPV-mediated carcinogenesis versus cigarette/alcohol-mediated carcinogenesis for oropharyngeal cancers. However, Smith found a *different* relationship between HPV, tobacco and alcohol for oral cavity cancers. The oral cavity cancer risk among heavy tobacco users was greater in HPV-seropositive patients (adjusted OR = 3.5) compared to HPV-seronegative patients (adjusted OR = 1.4); among heavy alcohol users the risk is also greater for HPV-seropositive patients (adjusted OR = 9.8) compared with HPV-seronegative patients (adjusted OR = 3.1) [101]. This interaction also suggests that profiling tumor suppressor gene mutational status in oral cancers will not result in the predicted dichotomy anticipated in oropharyngeal cancers (HPV+/wild type p53/wild type Rb vs. HPV−/mutated p53/mutated Rb).

Importantly, no data currently supports the idea that HPV is significantly associated with improved outcome for patients with oral cancer. The studies by Kaminagakura [51] and Sugiyama [89] do reveal nonsignficant trends, towards improved survival for patients with HPV-positive cancers. Therefore, future studies on oral cavity SCC should be powered to address the important clinical issue of HPV status (as determined by PCR).

HPV and Laryngeal Cancer

Fewer studies have addressed the association of HPV in laryngeal cancer [113, 116, 117]. Kreimer reviewed 35 PCR-based studies on 1,435 patients with laryngeal SCC and determined the *cumulative pooled prevalence* for HPV to be 24.0 %, (95 % CI 21.8, 26.3) [113]. We have found the WP of HPV is 23.9 %, (range 0–100 %, 95 % CI 17.1 %, 30.9 %) in laryngeal cancers. Hobbs determined the OR for laryngeal cancer is 2.0 (95 % CI 1.2, 3.4) as compared to controls. The most common HPV type detected in laryngeal cancers is HPV16; HPV18 is the second most common HPV type. However, compared to oral carcinomas, a greater diversity of other HPV types has been detected in laryngeal cancers. Low-risk HPV are uncommonly detected, but cannot be summarily dismissed as "bystander" infections as integrated low-risk HPV has been found in laryngeal cancers.

Very few studies have addressed the issue of causation regarding HPV and laryngeal cancer. HPV transcriptional activity was addressed in a total of nine cancers from three studies [2, 21, 81]. Duray demonstrated that the HPV16 viral load in laryngeal cancers (median 504 copies) was significantly higher than in benign lesions (median 37 copies). This supports the idea of active HPV "driver" infection, and suggested viral-mediated carcinogenesis. Future studies should address the issue of HPV RNA in laryngeal SCC.

P16 expression status should also be studied in the context of laryngeal cancer, with the same recommendations as above (whole tissue sections, documenting intensity and distribution). Baumann studied a subgroup of 10 laryngeal SCC including 5 HPV+ SCC and demonstrated good correlation [20]. Likewise, Laco also demonstrated good correlation between p16 overexpression and HPV status in 24 laryngeal SCC, 14 of which were HPV+ by chromogenic in situ hybridization (CISH) [118].

Importantly, only four studies [8, 9, 29, 88, 95] examined the impact of HPV on the outcome of a total of 319 patients; 134 of which were HPVpositive. No association of HPV status with outcome was found. Therefore, future studies on laryngeal SCC should be powered to address the important clinical issue of HPV status (as determined by PCR) and association with clinical outcome.

HPV and Sinonasal Cancer

The relationship between HR-HPV promoting malignant progression of inverted papillomas is well established. Future studies should focus on sinonasal SCC *specifically unrelated to IP*. Good correlation between HPV+ status, detected by either PCR or ISH, and diffuse p16 overexpression, has been reported in sinonasal SCC, albeit in small numbers [97, 119–121]. Strong diffuse p16 expression cannot be accepted as a surrogate HPV biomarker in any untested context, as this pattern of overexpression has also been detected in HPV-negative sinonasal undifferentiated carcinoma [122]. The improved outcome associated with HPV+ sinonasal SCC reported by Alos and colleagues [97] necessitates validation by other groups.

HPV and Nasopharyngeal Cancer

While recent studies suggest a exclusionary dichotomy between HPV-mediated NPC and EBV-mediated NPC [98–100, 102] NPC with double EBV/HPV infections have been reported [108]. An important caveat to future studies is the necessity for stringent exclusion of "NPC" which may have arisen from the oropharynx. With respect to HPV as a driver infection for NPC, three studies have demonstrated good correlation between HPV+ NPC and p16 expression, albeit on a small number of patients [98, 99, 107].

Nowhere is the possibility of "treatment de-escalation" more important that in the situation of skull base radiation, where the treatment related toxicities of optic nerve damage and osteoradionecrosis are most fearsome. Adequately powered, stringent studies comparing outcomes for patients with HPV-mediated NPC, EBV-mediated NPC, double-infected NPC, and "null" viral NPC may be challenging due to the relative rarity of NPC in western populations. However, these studies could have tremendous clinical impact.

HPV and Salivary Tumors

The emerging data demonstrate that HPV is detected in some benign and malignant salivary tumors. We have also mentioned our preliminary data on HPV16/18 E6/E7 RNA in MEC. Correlation of any biomarker or grading schema, with outcome for patients with salivary malignancies is extremely challenging given the overall rarity of even "common" salivary malignancies and the need for even larger sample sizes on multivariate analysis to account for a greater number of possible anatomic tumor sites.

In conclusion, high-risk HPV DNA is present in a significant proportion of oral and laryngeal cancers. There is limited published data on HPV-positive oral and laryngeal carcinomas regarding RNA expression, physical state (episomal vs. integrated), and correlation with tumor suppressor gene mutational status. Therefore, a causative relationship between HPV and these nonpharyngeal cancers has not yet been firmly established. Importantly, only few studies have attempted to correlate HPV status with

clinical outcome. This review justifies the need for additional, appropriately powered, well-designed studies to examine the relationship of HPV status and clinical outcome for patients with oral and laryngeal cancer. High risk HPV DNA is also present in a significant proportion of sinonasal, nasopharyngeal, and salivary gland cancers, but the clinical significance of these findings in these malignancies has yet to be clearly defined.

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