Human papillomavirus and head and neck cancer: a systematic review and meta-analysis

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Keypoints

- It has been suggested that the link between human papillomavirus (HPV) and head and neck squamous cell carcinoma (HNSCC) is specific to carcinoma of the tonsil.
- We systematically reviewed studies that tested for HPV16 exposure in anatomically defined sites in the head and neck and a control group.
- The association between HPV16 and cancer was strongest for tonsil (OR: 15.1, 95% CI: 6.8–33.7), intermediate for oropharynx (OR: 4.3, 95% CI: 2.1–8.9) and weakest for oral (OR: 2.0, 95% CI: 1.2–3.4) and larynx (OR: 2.0, 95% CI: 1.0–4.2).
- To investigate heterogeneity, further stratification by method of HPV16 detection, suggested that variation in the magnitude of the HPV-cancer association with cancer site was restricted to studies using ELISA: among studies using PCR, the magnitude of the summary odds ratios was similar across the four sites.
- The association between HPV16 infection and HNSCC in specific sites suggests the strongest and most consistent association is with tonsil cancer, and the magnitude of this association is consistent with an infectious aetiology.
- However, the method of viral detection may be an important source of heterogeneity. Resolution of this issue will require further studies using both methods, examining associations separately in different sites.

A link between human papillomavirus (HPV) and squamous cell carcinoma of the head and neck (HNSCC) was suggested >20 years ago.[1] HPV DNA has been isolated from tumours throughout the upper aero-digestive tract, with a wide variation in prevalence. There appears to be a stronger link with a subset of head and neck cancers, namely those arising from the oropharynx. A recent systematic review of case-series using PCR-based methods to detect and genotype HPV in head and neck cancer biopsies, showed significantly higher HPV prevalence in oropharyngeal SCCs than oral cavity or laryngeal SCCs.[2] It has been suggested that HPV-positive oropharyngeal cancers comprise a distinct molecular, clinical and pathologic disease entity that are likely to be causally associated with HPV infection[3] and are less dependent on traditional risk factors such as smoking and alcohol.[4]

Questions remain, however, in particular whether HPV is causally associated with cancer development at other sites in the head and neck. We systematically reviewed observational studies investigating HPV16 exposure in head and neck cancer, to test the hypotheses that HPV risk varies according to anatomical site within the upper aero-digestive tract; and that variation is independent of the method used to detect the virus.

Materials and methods

Studies were located using systematic searches in Medline, Embase, Cinahl, and the Cochrane Library electronic databases (from inception to February 2004), together with hand searching of key texts and reviews that were highly relevant to the subject field. A hierarchical literature search in Medline used the following National Library of Medicine Medical Subject Headings: 'Head and Neck Neoplasms'; 'Papillomavirus, Human'; 'Larynx'; 'Pharynx' and 'Mouth.' The additional keywords oral, tonsil, oropharynx, hypopharynx and cancer/carcinoma were also used in Medline (truncated where necessary) and in searching the remaining databases.

Eligible studies were those that had tested for HPV16 exposure in anatomically defined sites [oral cavity (ICD

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C00.3,4 06.0,1,2 03.0,1 04.0,1,8 05.0 02.0,1,2,8), tonsil (ICD 09.0,1,9), oropharynx (ICD-0 C01, C05.1,2, C10.0,2,3,9) or larynx (ICD C32.0,1,2,3,9)] in cases with histologically defined squamous cell carcinoma as well as in a control group. To identify potentially eligible studies the title and abstract of each study identified by the literature search were assessed independently by two authors. Full papers for these studies were also reviewed in duplicate. Disagreements were resolved by discussion. Data extracted included numbers of cases and controls who were and were not infected with HPV, year of publication, country, sample size, anatomical site of tumour, methods used to obtain tissue for HPV detection and methods used to detect the virus.

Analysis

For unmatched case–control studies, we derived the log odds ratio and its standard error for each cancer site in each study. Two studies undertook a matched analysis.[5,6] For the study by Mork *et al.*,[5] we derived an overall odds ratio for the association of HPV16 with oral cancer by meta-analysing the published crude-matched analysis for cancer of larynx, oropharynx, tongue and 'oral cavity not otherwise specified' together with the estimated association of HPV16 with cancer floor of mouth calculated from the raw numbers published in the paper (unmatched analysis). This was because a matched analysis for floor of mouth was not reported in the paper. Dahlstrom *et al.*[6] kindly supplied us with separate estimates of the association of HPV16 with cancer of the tonsil, oropharynx excluding tonsil, oral cavity and larynx.

We used inverse-variance weighted fixed-effect meta-analysis, and DerSimonian and Laird random-effects meta-analyses[7] to derive summary odds ratios according to cancer site and to the method of detecting HPV (PCR or ELISA). Between-study heterogeneity was measured using I^2 statistics.[8] Differences in the magnitude of the association according to method of detection were examined using random-effects meta-regression.[9] Stata version 8.2 was used for all analyses.

Results

Twenty studies that compared site-specific head and neck cancer with a control group were identified. Three of these were excluded because it was not possible to separate the data for specific anatomical sites for cancer cases.[10–12] Therefore, seventeen studies were included in the review.[5,6,13–27] These provided a total of 2612 cases of head and neck cancer: 1656 oral cavity, 383 oropharynx, 161 tonsil (distinct from oropharynx) and 412 larynx. Because four of the studies examined HPV16 exposure only, we restricted all analyses to this genotype.

Study characteristics

Table 1 shows characteristics of the studies including the tissue sampled and numbers of cases and controls with proportions positive for HPV16. The method for detecting HPV16 was enzyme-linked immunosorbent assay on serum samples in five studies (measuring HPV16 L1 antibody),[5,6,17,25,26] DNA amplification in ten studies[15,16,18–24,27] and *in situ* hybridisation[14] and Southern blot[13] in the remaining studies. The majority used cases identified from hospital records with serum or biopsies from non-consecutive controls acquired in the hospital setting. In two studies, cases were identified from national cancer registries linked to large populations with banked serum. Controls were selected from the same populations.[5,17]

HPV detection

The prevalence of HPV16 according to case–control status, site of the cancer, the tissue examined and the method used to detect the virus are shown in Table 1. The proportion of head and neck cancer cases positive for HPV16 ranged from 0% to 86% and from 0% to 38% in controls. HPV16 DNA was isolated in 94.7% of HPV positive cases by Herrero *et al.*[25], other genotypes were far less commonly identified and when present usually involved a mixed infection with HPV16. HPV detection was more likely in those with lifetime number of sexual partners greater than one (OR: 2.4, 95% CI: 1.0–5.7) or who practised oral sex (OR: 3.2, 95% CI: 1.5–6.4).[25] Six studies recorded tumour grade, with only one showing a significant correlation between HPV positive cases and poor differentiation.[22]

Interaction with cofactors

Potential confounding factors were considered in six studies (Table 2). These studies reported effect estimates adjusted for smoking amongst other potential confounders. There was some evidence that adjusting for smoking increased the strength of the association between HPV and head and neck cancer. The estimates for tonsil and oropharynx excluding tonsil, before and after adjustment for smoking, were calculated in a secondary analysis by Dahlstrom *et al.*[6] at the authors request.

Analysis

Meta-analysis stratified by anatomical site (Fig. 1) suggested that the association between HPV and cancer was

Table 1. Characteristics of eligible studies examining the association of HPV16 with cancers of the head and neck

Reference	Site	Case/cont	% HPV		Cases tissue	Control tissue	HPV detection including type specific
			Positive case	Positive controls			
Brandsma and Abramson[13]	Oral	21/18	10	0	Biopsy	Biopsy	Southern blot
	Larynx	60/53	5	4	Biopsy	Biopsy	Using probes
	Tonsil	7/20	29	0	Biopsy	Biopsy	11, 16, 18
Niedobitek *et al.*[14]	Tonsil	28/30	21	0	Biopsy*	Biopsy	In situ hybridisation 6, 11, 16
Snijders *et al.*[15]	Tonsil	10/7	50	0	Biopsy	Biopsy	GP5/GP6 primers HPV 6, 11, 16, 18, 31, 33
Lewensohn *et al.*[16]	Tonsil	4/2	50	0	Biopsy	Biopsy	MY09/MY11/GP5/GP6 primers HPV 6, 16, 18, 31, 33
Dillner *et al.*[17]	Oral	29/–	3	0	Serum	Serum	HPV16 L1 ELISA
	Larynx	37/–	3	0	Serum	Serum	
Garcia-Milian *et al.*[18]	Larynx	32/22	47	5	Biopsy	Biopsy	MY09/MY11 primers Southern blot HPV 6, 11, 16, 18 Type specific PCR HPV 16/18
Nishioka *et al.*[19]	Oral	15/7	0	0	Biopsy	Biopsy	HPV 16, 18 if
	Larynx	27/35	19	6	Biopsy	Biopsy	+ve on MY09/11
Mellin *et al.*[20]	Tonsil	60/10	43	0	Biopsy*	Biopsy	GP5+/GP6+ primers HPV 16,33
Smith *et al.*[21]	Larynx	39/12	10	0	Biopsy	Mouth wash	MY09/MY11 primers DNA sequence analysis
Klussmann *et al.*[22]	O/pharynx	33/14	45	0	Biopsy	Biopsy	A10/A5 & A6/A8 CP62/70 &
	Tonsil	24/14	58	0	Biopsy	Biopsy	CP65/69a, sequencing of PCR products + HPV16 specific PCR
Mork *et al.*[5]	Oral	99/531	11	7	Serum	Serum	HPV 16 L1/L2 ELISA
	Larynx	76/411	12	5	Serum	Serum	HPV 18 L1/L2 ELISA
	O/pharynx	26/137	38	10	Serum	Serum	HPV 33 L1/L2 ELISA HPV 73 L1 ELISA
Chen *et al.*[23]	Oral	28/25	86	32	Biopsy*	Biopsy	MY09/MY11 & type specific In situ hybridisation HPV 6, 11, 16, 18
Strome *et al.*[24]	Tonsil	52/48	40	6	Biopsy*	Biopsy	MY09/MY11, sequencing & type specific PCR HPV 16 Southern blot in control biopsies
Dahlstrom *et al.*[6]	Oral	36/36	8	9	Serum	Serum	HPV16 L1 ELISA
	Larynx	14/14	36	9	Serum	Serum	
	O/pharynx†	38/38	58	9	Serum	Serum	
	Tonsil	32/32	59	9	Serum	Serum	
Herrero *et al.*[25]	Oral	1299/1527	9	6	Serum	Serum	HPV16 L1, E6, E7 ELISA‡
	O/pharynx	238/1527	13	6	Serum	Serum	
Van Doornum *et al.*[26]	Oral	56/100	21	18	Serum	Serum	HPV16 L1 ELISA‡
	Larynx	127/100	20	18	Serum	Serum	HPV16E7 ELISA
	O/pharynx	48/100	33	18	Serum	Serum	
Zhang *et al.*[27]	Oral	73/40	68	38	Biopsy*	Biopsy	Type specific PCR HPV 16/18

*Archival paraffin-embedded samples.
†Oropharynx excluding tonsil.
‡HPV16 L1 data only used to enable comparison with the other studies.

strongest for tonsil (random-effects summary OR: 15.1, 95% CI: 6.8–33.7), intermediate for oropharynx (random-effects summary OR: 4.3, 95% CI: 2.1–8.9) and weakest for oral (random-effects summary OR: 2.0, 95% CI: 1.2–3.4) and larynx (random-effects summary OR: 2.0, 95% CI: 1.0–4.2). There was evidence of between-study heterogeneity in these effects for oral cancer ($I^2 = 61.7\%$, $P = 0.01$), larynx cancer ($I^2 = 47.0\%$, $P = 0.07$) and oropharynx cancer ($I^2 = 57.0\%$, $P = 0.05$) but not for tonsil cancer ($I^2 = 0$, $P = 0.98$). Within the larynx, two studies examined the risk of supraglottic *versus* glottic cancer with HPV infection: sample sizes were small and the direction of effect opposite in the two studies.[6,21]

Further stratification by method of detection of HPV (excluding the two studies that used *in situ* hybridisation or Southern blot) suggested that the variation in the

Table 2. Minimally adjusted (or crude) odds ratios and maximally adjusted odds ratios and 95% confidence intervals where reported for the association of HPV16 with cancers of the head and neck

Author	Confounding factors	Site	Odds ratio (95% CI)	
			Minimally adjusted	Adjusted
Herrero *et al.*	Country gender, age, smoking, alcohol, paan chewing	Oral cavity	1.5 (1.2–2.0)	1.5 (1.1–2.1)
		Oropharynx	2.5 (1.6–3.8)	3.5 (2.1–5.9)
Dahlstrom *et al.*	Cotinine, alcohol	Oropharynx	38.0 (5.2–276.8)	59.5 (5.7–620.2)
		Head and neck	5.8 (2.7–12.2)	6.7 (3.0–14.9)
Chen *et al.*	Gender, age, smoking	Oral cavity	12.8 (3.3–49.3)	11.2 (1.2–103.2)
Strome *et al.*	Gender, age, smoking	Tonsil	18.2 (4.6–73.1)	42.6 (6.6–273.4)
Mork *et al.*	Cotinine	Tongue	2.7 (1.2–6.4)	2.8 (1.2–6.6)
		Oral cavity	5.4 (0.8–38.8)	3.6 (0.5–26.3)
		Oropharynx	8.6 (2.6–28.5)	14.4 (3.6–58.1)
Dillner *et al.*	Smoking	Oral cavity	0.6 (0.1–7.4)	0.4 (0.0–7.1)
		Larynx	0.2 (0.0–1.6)	0.2 (0.0–2.0)

magnitude of the HPV-cancer association with cancer site was restricted to studies using ELISA (Fig. 2). Among the studies using PCR, the magnitude of the summary odds ratios was similar across the four sites, with overlapping confidence intervals. However, it should be noted that only one study of tonsil cancer used antibody detection, and similarly only one study of oropharyngeal cancer used PCR. Oral cavity and larynx had multiple studies using both methods. For these sites, the ratio of odds ratios (ROR) comparing antibody with PCR studies (estimated using random-effects meta-regression) was 0.3 (95% CI: 0.1–0.7) for oral cancer and 0.2 (95% CI: 0.04–1.3) for larynx cancer. Corresponding comparisons for the other cancers resulted in wide confidence intervals [oropharynx ROR: 0.2 (95% CI: 0.01–6.3); tonsil ROR: 2.2 (0.3–16.6)].

Discussion

This review of studies that assessed the association between HPV16 infection and head and neck cancer in specific sites, suggests an increased risk at each of the sites examined but the strongest and most consistent association is with tonsil cancer, and the magnitude of this association is consistent with an infectious aetiology.

The prevalence of HPV16 varied widely among cases (0–86%) and among controls. The tissue examined varied as did the method used to detect the virus. The method of detection of HPV16 may be an important source of heterogeneity in the results of these studies. PCR techniques are very sensitive and in the absence of a quantitative method such as Real-Time PCR, insignificant virus (with no role in carcinogenesis) can be identified. In contrast, ELISA lacks sensitivity in detecting clinically meaningful HPV infections.

Those that detected HPV16 using serum ELISA were consistent with the idea that HPV is a strong risk factor for SCC in tonsil but less important elsewhere. There was less evidence of between-site heterogeneity in the association between HPV and cancer, in studies using amplification of HPV nucleic acid. Direct comparisons of results according to method of detection suggested that for oral, larynx and oropharynx cancer, the association with HPV was greater in studies that used DNA amplification. However the majority of studies were small, and so these trends must be interpreted with caution.

Our study was based on a systematic search of the published literature, with eligibility assessed by two independent observers. Only a minority of studies provided estimates controlled for the effect of confounding factors: these suggested that control for smoking generally increased the magnitude of the association between HPV and head and neck cancer. Although, the characteristics of the controls are unlikely to be critical where a powerful biological influence is present, as appears to be the case for HPV, selection bias cannot be excluded because most studies used unmatched non-consecutive controls.

Misclassification of anatomical site is of concern. The main risk factors in the Taiwanese population described by Chen *et al.* are *Areca catechu* chewing ('betel nut') and smoking, which are more likely to give rise to tumours of the oral cavity rather than oropharynx. Herrero *et al.*[25] included cancers topographically classified as 'base of tongue' with oral cavity cancers while only cancers

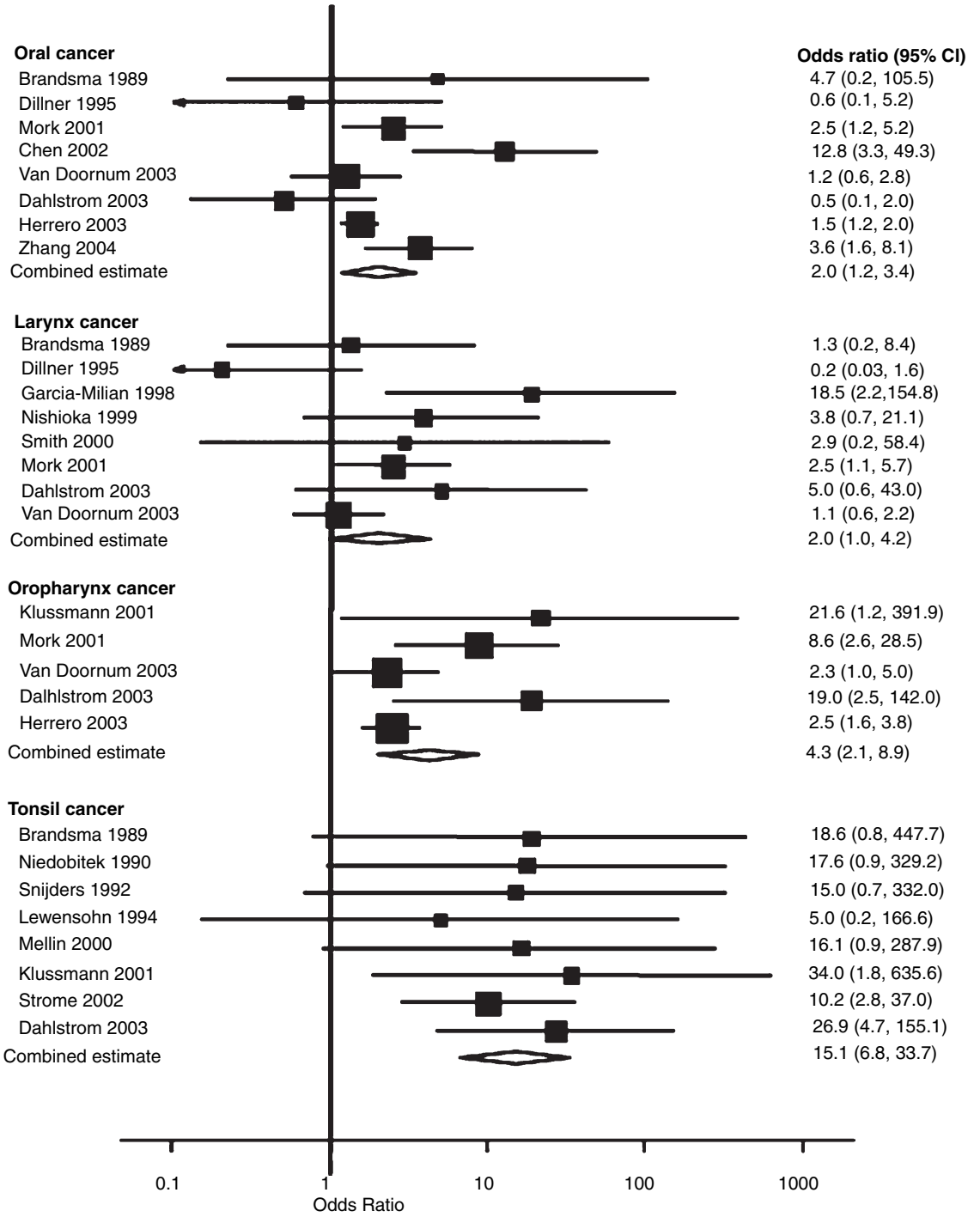

Fig. 1. Association of head and neck squamous cell cancer with HPV16 infection, according to cancer site.

topographically classified as 'oropharynx' or 'tonsil' composed their oropharynx group. Misclassification of tongue base as oral instead of oropharyngeal cancer could have led to an overestimate of risk of oral cancer with HPV and could explain some of the heterogeneity found in this group. Furthermore, supraglottic larynx may have a different rate of positivity than glottic larynx; however, only two studies discriminated between these sites and the number of observations are small; the risk of HPV positivity in glottic *versus* supraglottic was increased one study[21] and reduced in the other.[6]

A number of recent studies,[25,28] reviews[2,29] and commentaries[3] have suggested, based on selected epidemiological and laboratory evidence, that HPV is a causative

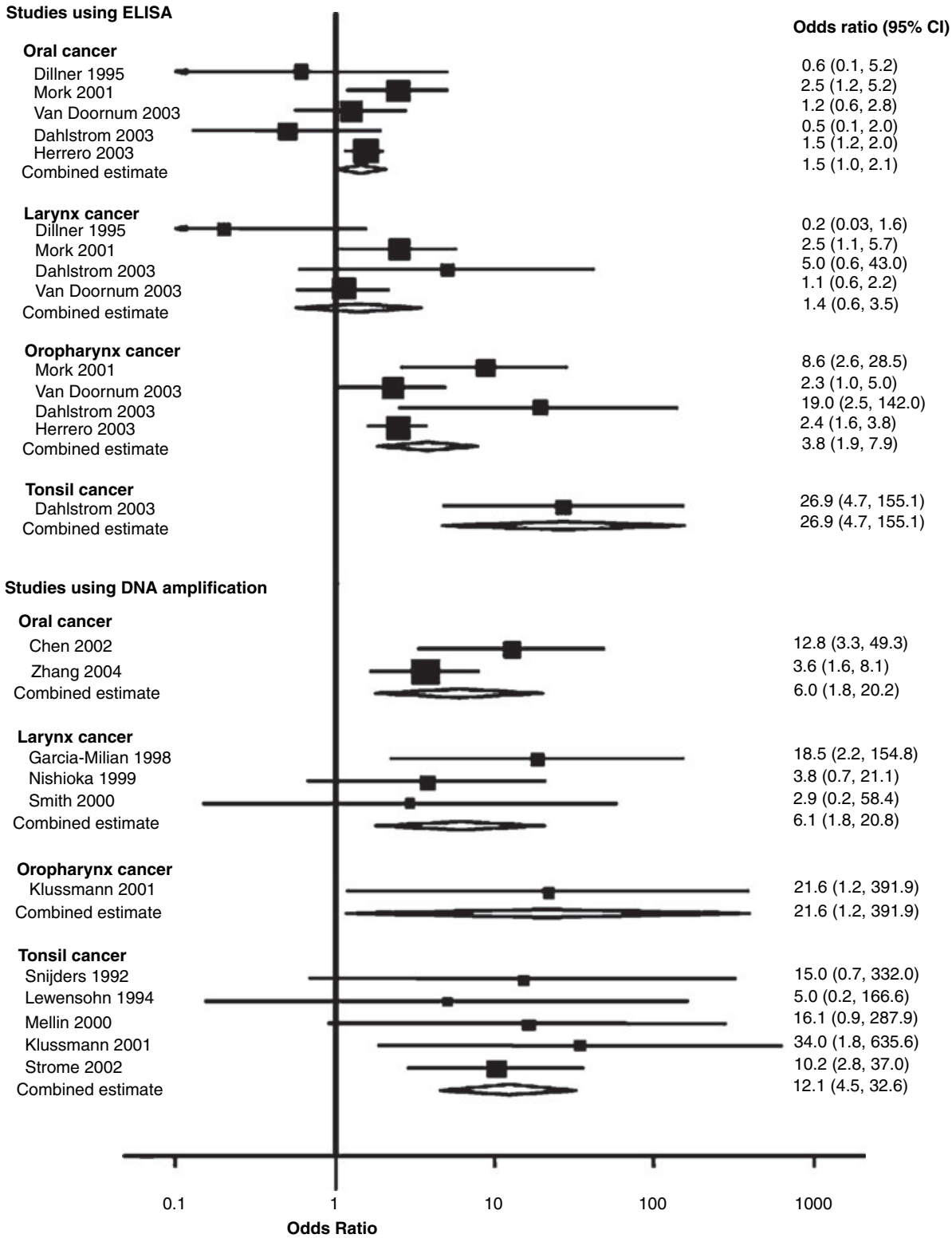

Fig. 2. Association of head and neck squamous cell cancer with HPV16 infection, according to cancer site and method of viral detection.

agent for some head and neck squamous-cell carcinoma, specifically those associated with the oropharynx, but not cancers elsewhere in the upper aero-digestive tract. Cited molecular evidence includes the expression of viral oncogenes[30] and a higher viral load[31] which appear to be specific to HPV-associated tonsil cancers.

While our systematic review leads us to question whether HPV has a specific role in tonsil SCC, the number of studies is small. The inconsistency between studies using ELISA and PCR may reflect differences in sensitivity and specificity between the two techniques. In addition, the apparent discrepancy in ELISA between oropharyngeal cancer and other sites, may result from the relationship between HPV infection and the immunological architecture of the upper aero-digestive tract. Systemic antibody is removed one step from the process of epithelial infection with HPV and the level and type of systemic antibody response will depend on a number of factors. Organised lymphoid tissue such as the tonsils are effective inductive sites for systemic as well as mucosal immune responses,[32] antigen presentation at this site may result in a different immune response to presentation at other sites in the upper aero-digestive tract.

There is a solution as to what constitutes a biologically relevant infection. Wiest *et al.*[33] suggest that a causal role for HPV16 in head and neck cancer can be defined by the presence E6/E7 mRNA, viral integration with an intact E6 gene and unaltered p53 status. In other words, a specific genetic profile of the tumour has been found when HPV16 is transcriptionally active, which can be interpreted as proof for an active viral involvement in the very early phase of carcinogenesis.[34] As a consequence, an assessment of the genetic pattern (loss of heterozygosity at the chromosomal arms) may allow the tumour to be classified as biologically HPV positive or biologically HPV negative. This is especially important when no good quality RNA is available. Similarly, E6 or E7 antibodies, which were not included in the meta-analysis because they were only measured in one study,[25] probably discriminate for HPV association more effectively than L1 antibody.

Conclusion

This review suggests that HPV is a risk factor for some head and neck cancers. The magnitude of the risk of tonsil carcinoma is consistently high and of the order expected with an infection aetiology. The role of HPV in tonsil cancer we have shown suggests that, like Epstein–Barr virus and nasopharyngeal cancer, this is another cancer site in pharyngeal lymphoid tissue with a viral aetiology. However, the conclusion that this association is exclusive to the tonsil or the more broadly defined oropharynx may be premature, as this pattern is seen mainly in studies based on ELISA, it could relate to methodology or result from a differential site-specific immune response. Resolution of this issue will require further studies using quantitative PCR, E6/E7 ELISA and gene expression analysis, examining associations separately in difference sites, and dealing adequately with confounding factors.

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Conflicts of interest

None declared.

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