Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: a systematic review

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Background: A significant proportion of squamous cell carcinomas of the oropharynx (OP-SCC) are related to human papillomavirus (HPV) infection and p16 overexpression. This subgroup proves better prognosis and survival but no evidence exists on the correlation between HPV and p16 overexpression based on diagnostic measures and definition of p16 overexpression. We evaluated means of p16 and HPV diagnostics, and quantified overexpression of p16 in HPV-positive and -negative OP-SCCs by mode of immunohistochemical staining of carcinoma cells.

Methods: PubMed, Embase, and the Cochrane Library were searched from 1980 until October 2012. We applied the following inclusion criteria: a minimum of 20 cases of site-specific OP-SCCs, and HPV and p16 results present. Studies were categorised into three groups based on their definition of p16 overexpression: verbal definition, nuclear and cytoplasmatic staining between 5 and 69%, and ≥70% staining.

Results: We identified 39 studies with available outcome data (n = 3926): 22 studies (n = 1980) used PCR, 6 studies (n = 688) used ISH, and 11 studies (n = 1258) used both PCR and ISH for HPV diagnostics. The methods showed similar HPV-positive results. Overall, 52.5% of the cases (n = 2062) were HPV positive. As to p16 overexpression, 17 studies (n = 1684) used a minimum of 5–69% staining, and 7 studies (n = 764) used ≥70% staining. Fifteen studies (n = 1478) referred to a verbal definition. Studies showed high heterogeneity in diagnostics of HPV and definition of p16. The correlation between HPV positivity and p16 overexpression proved best numerically in the group applying ≥70% staining for p16 overexpression. The group with verbal definitions had a significantly lower false-positive rate, but along with the group applying 5–69% staining showed a worse sensitivity compared with ≥70% staining.

Conclusions: There are substantial differences in how studies diagnose HPV and define p16 overexpression. Numerically, p16 staining is better to predict the presence of HPV (i.e. larger sensitivity), when the cutoff is set at ≥70% of cytoplasmatic and nuclear staining.

Oral and pharyngeal cancers are the sixth most frequent tumour with over 482 000 new cases and 273 000 deaths worldwide in 2008 (Ferlay *et al*, 2010). The role of high-risk human papillomavirus

(HR-HPV) in the carcinogenesis of the uterine cervix is well recognised (Bosch *et al*, 1995), and owing to numerous studies in the past 10 years, HR-HPV is now also a well-known risk factor in

oropharyngeal squamous cell carcinomas (OPSCCs) in addition to established factors such as tobacco and alcohol exposure (Dayyani *et al*, 2010). Compared with other head and neck squamous cell carcinomas (HNSCCs), HPV-related OPSCCs have different epidemiology, histopathological characteristics, therapeutic response, and clinical outcome (Shah and Patel, 2003; Fakhry and Gillison, 2006; De Vita *et al*, 2008; Robinson *et al*, 2010; Westra, 2012).

The small, non-enveloped, DNA virus HPV belongs to the Papillomaviridae family and is known commonly to infect squamous epithelial cells (Doorbar *et al*, 2012). Cell morphology alone is insufficient to determine the presence of HPV (Lewis *et al*, 2012), although HPV-positive oropharyngeal cancers are often characterised histologically by a non-keratinising or basaloid morphologic pattern. Two techniques are generally used to diagnose HPV: polymerase chain reaction (PCR) and *in situ* hybridisation (ISH). Both have strengths and limitations. Human papillomavirus-specific PCR is not routinely available in most diagnostic laboratories; few HPV PCR tests are approved for clinical use, and the method requires a high level of technical skills and special laboratory facilities to prevent contamination. When applied to extracts made from fresh-frozen biopsy samples, the highest sensitivity is obtained, but the PCR analysis does not distinguish the mere presence of HPV from a clinically relevant HPV infection, where the HPV genome is often integrated into the host genome and actively transcribes HPV oncoproteins. Detection of HPV with ISH provides evidence of viral genomes through mRNA or DNA present in the tumour nuclei and is highly specific, although less sensitive than PCR (Robinson *et al*, 2010). This method does not differentiate between integrated and non-integrated genomes.

The presence of HR-HPV DNA is insufficient to classify accurately tumours as an HPV infection as it may be biologically inactive and not the cause of malignancy. Along with HPV diagnostics, immunohistochemical detection of p16 (p16-IHC) is often used as a surrogate marker for HPV infection and an activity of viral oncoproteins. P16 is a tumour suppressor gene that inhibits cyclin-dependent kinase 4A. In the presence of transcriptionally active HPV, hypophosphorylated retinoblastoma protein (pRb) bind to the HPV oncoprotein E7, allowing the transcriptional activator E2F to be constitutionally active while effectively stopping the negative feedback of free pRb on p16. Overexpression of p16 ensues. Independent of treatment modality, OPSCC patients with p16 overexpression have better prognosis and clinical outcome (Langendijk and Psyrri, 2010). P16-IHC is generally accessible and its technical costs are estimated to be 2–16 times lower than other HPV-specific tests (Lewis, 2012). Several studies have reported difficulties in HPV and p16 diagnostics, as there is no consensus on defining overexpression of p16 by a clear percentage cutoff level, and definitions vary from ⩾5%, ⩾75% to numerous less specific verbal definitions, for example, 'diffuse and strong nuclear and cytoplasmatic staining' (Smeets *et al*, 2007; Lewis, 2012). This may be problematic because different staining patterns can correlate differently to HPV-positive and -negative tumours, and staining patterns may ultimately distinguish transcriptionally from non-transcriptionally active HPV infections and thereby help determine prognosis and clinical outcomes.

The aim of this systematic review was to define and categorise overexpression of p16 based on immunohistochemical staining and correlate the categories to HPV-positive and -negative OP-SCCs.

Embase, and the Cochrane Library. The search strategy was as follows including MESH terms and keywords: 'HPV' or 'papillomavirus' or 'papillomaviridae' and 'p16' or 'cdkn2a' or 'cyclin-dependent kinase inhibitor p16' or 'p16 genes' and 'oropharynx' or 'oropharyngeal' or 'palatine tonsil' or 'tonsil' or 'palatine' or 'tongue' or 'mouth' or 'oral'. Two authors (CGL and MG) independently reviewed the relevance of all resulting study titles and abstracts identified through the above search, and full-text copies of potentially eligible articles were assessed. Finally, one author (CGL) reviewed reference lists of the initially included studies. Studies with identical authors were contacted to avoid including the same study population twice.

We included all studies published in English from January 1980 to October 2012 regardless of funding source. The inclusion criteria were restricted to: age above 18 years, a minimum 20 cases of site-specific OP-SCCs (morphologic variants were included), and HPV and p16 results stated.

Data synthesis. Two authors (CGL and MG) independently extracted relevant data from the included studies and entered them into a piloted data extraction form. The following information were recorded: country, year(s) of biopsy collection, demographics, number of cases, tumour site (base of tongue, palatine tonsils, or other), tumour morphology (keratinising, non-keratinising, or mixed), histopathological grade (carcinoma *in situ*, poor, moderate, or high differentiation), IHC staining probe, definition of p16 overexpression, biopsy preservation (fresh frozen or paraffin embedded), IHC evaluation by pathologists (yes or no), HPV results (negative or HPV-16, HPV-18, HPV-33, HPV-35, and HPV-58 positive), HPV diagnostics (HPV DNA PCR, HPV DNA ISH, and HPV DNA ISH followed by PCR, HPV RNA RT–PCR, and HPV RNA ISH), and the number of p16-positive and negative cases.

Included studies were categorised into three groups by their definition of p16 overexpression: (a) a verbal definition (e.g. 'Cases were classified in a binary manner as either positive (any cells with nuclear and cytoplasmatic staining) or negative'), (b) 5–69% nuclear and cytoplasmatic staining, and (c) ⩾70% staining.

Statistical analysis. Statistics were carried out using IBM SPSS Statistics 19.0 (IBM SPSS, Chicago, IL, USA). Descriptive statistics are presented as actual numbers and percentages, or median and range where appropriate. We conducted a meta-analysis using the bivariate model (Reitsma *et al*, 2005). In the bivariate model, the logit-transformed sensitivities and specificities and the correlation between them across studies are modelled directly. The model accounts for sampling variability within studies and also account for between-study variability through the inclusion of random effects. In the preliminary meta-analyses for each definition of p16 positivity, we fitted the bivariate model separately for each test, and obtained a diagnostic odds ratio, sensitivity, and specificity. Hierarchical summary receiver-operator curve (HSROC) was applied in the meta-analysis and is recommended in the current meta-analytic literature for diagnostic meta-analyses (Leeflang *et al*, 2013). In addition, HSROCs were plotted with 95% CI. Afterwards, we compared the tests in two separate models, where the definitions used were included as covariates in a meta-regression. Variance components were estimated by restricted maximum likelihood, because of the number of studies and the heterogeneity of the included studies. Statistical analyses on meta-regression were performed in R using the mada package function reitsma.

METHODS

Search strategy and selection criteria. One author (CGL) undertook electronic literature searches within PubMed (Medline),

RESULTS

The initial literature search yielded a total of 778 records. From these, we manually selected 160 articles for full-text assessment,

of which 112 articles were later excluded. Accordingly, 48 studies were left eligible for inclusion (Figure 1). Additional three studies were later identified through searching reference lists. Studies with identical authors were contacted and resolved in 12 studies excluded; 11 studies were confirmed duplicates by authors; and one study excluded without reply from authors. Thus, a total of 39 studies ($n = 3926$) were included in the review (Table 1).

In the pooled analysis of all studies with demographic information ($n = 3625$), the majority of patients were male subjects ($n = 2921$, 80.6%). Age ranged from 20 to 93 years with a median of 58 years. Thirty-four studies ($n = 3420$ subjects) were European, Australian, or US based, and five studies ($n = 506$ subjects) were Asian. Ethnicity was reported in 22 studies ($n = 2265$), with 69.2% of these patients being Caucasian ($n = 1568$), 11.9% ($n = 269$) were of Asian origin, and 18.9% ($n = 428$) had mixed ethnicity. Tumours were represented throughout the oropharynx, but were primarily located in the palatine tonsils ($n = 1420$, 36.2%). Tumours at the base of the tongue ($n = 414$, 10.5 %) and of unspecified location represent the remaining ($n = 2092$, 53.3%) (Table 2).

A total of 52.5% cases ($n = 2062$) were found HPV positive by PCR, ISH, or both. For HPV diagnostics, 22 studies ($n = 1980$) used PCR, 6 studies ($n = 668$) used ISH, and 11 studies ($n = 1258$) used both techniques. In the PCR-based HPV-testing group, 49.6% ($n = 984$) of cases were said to be positive and 59.8% of cases ($n = 412$) were positive in the ISH group, whereas 52.9% ($n = 666$) were positive when both diagnostic approaches were used. The definition of p16 overexpression varied, but all studies dichotomised the results to either negative or positive. In the pooled analysis, p16 overexpression was shown by 37.6% ($n = 1478$) of subjects based on a verbal definition, by 42.9% ($n = 1684$) of subjects based on staining between 5 and 69%, and finally, by 19.5% ($n = 764$) of subjects based on staining equal to or exceeding 70% (Table 2).

Centres placed in the United States defined p16 as positive when staining was between 5 and 69% (6 centres, $n = 770$) or based on staining equal to or exceeding 70% (4 centres, $n = 482$). Six centres ($n = 507$) used a verbal definition. European centres either defined p16 as positive when staining was between 5 and 69% (9 centres, $n = 602$) or based on staining exceeding 70% (3 centres, $n = 282$).

Four centres ($n = 562$) used a verbal definition. Three centres ($n = 194$) in Asia used a verbal definition, and two centres ($n = 312$) defined p16 as positive when staining was between 5 and 69%. No Asian centres defined p16 as positive based on staining equal to or exceeding 70%.

Eleven studies ($n = 861$) reported data on histopathologic grade (poorly differentiated, moderate differentiated, highly differentiated, or carcinoma *in situ*), and six studies ($n = 634$) reported status on tumour morphology (keratinising, non-keratinising, mixed, or unknown). The limited availability of data on tumour morphology did not allow us to examine systematically to what degree the non-keratinising tumours were related to the presence of HPV, as has been observed previously. We found no trends regarding publication year and definition of p16, likely owing to the fact that the included studies were all published in the past 10 years.

Twenty-five studies ($n = 2888$) provided sufficient information to construct a two-by-two table of both p16-negative/-positive and HPV-negative/-positive biopsies. The correlation between HPV and p16 overexpression was numerically greater, when positivity was defined as staining above $\geqslant 70\%$ with a sensitivity of 0.927 (95% CI: 0.793–0.974). The verbal group and $>5–<70\%$ group had a sensitivity of 0.791 (95% CI: 0.608–0.888) and 0.894 (95% CI: 0.805–0.942), respectively. The false-positive rate of 0.059 (95% CI: 0.031–0.112) for the verbal group was superior to the rate of 0.201 (95% Cl: 0.12–0.337) of p16 $\geqslant 70\%$ (see Figure 2).

DISCUSSION

This is the first systematic review exploring the correlation between HPV infection and p16 overexpression in OPSCCs. This review shows that p16 overexpression correlates numerically better to HPV results if staining of tumour cells exceeds 70% rather than lower percentages or positivity based on a verbal definition. The issue of determining a specific cutoff value for p16 positivity has earlier been addressed in smaller samples supporting staining above 75% or staining above 50% combined with $>25\%$ confluent areas to define p16 positivity (Begum and Westra, 2008). We found
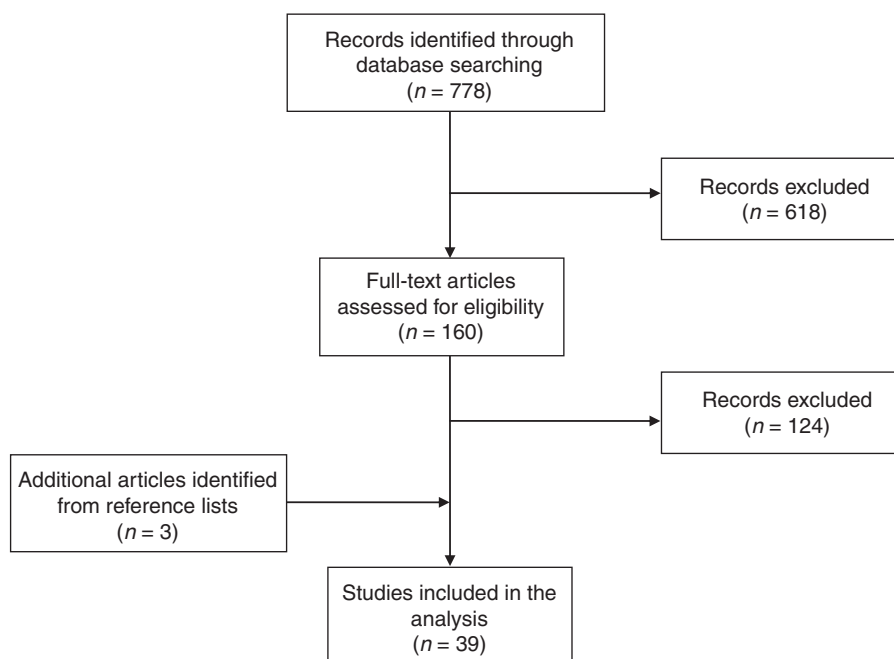
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│  Studies included in the │
│        analysis          │
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Figure 1. PRISMA diagram.

Table 1. Overview of studies

Author (year)	Country	Year	Study years	n	Age (years)	M/F	HPV+	Analysis	P16+	P16 cutoff (%)	P16 antibody	Sensitivity of p16 (%)	Specificity of p16 (%)
Al-Swiahb et al (2010)	Taiwan	2010	1992–2008	220	51	206/14	33	PCR	36	≥5% and <70	Clone unknown (Neomarkers, Fremont, CA, USA)	86	99
Ang et al (2010b)	USA	2010	2002–2005	323	Unknown	271/52	206	PCR	214	≥70	E6H4 (MTM Laboratories AG, Heidelberg, Germany)	90	93
Charfi et al (2008)	France	2007	1987–2005	52	61	36/16	32	PCR	25	≥5 and <70	E6H4 (MTM Laboratories AG, Heidelberg, Germany)	84	59
Chenevert and Chiosea (2012)	USA	2012	1956–1969 and 2007–2009	97	58	80/17	54	ISH	57	≥70	G175-405 (BD Pharmingen, San Diego, CA, USA)	69 (second period: 86%)	80 (second period: 90%)
El-Mofty and Patil (2006)	USA	2006	Unknown	20	Unknown	Unknown	12	ISH	11	Verbal definition	Clone unknown (Novacastra Labs Ltd, Newcastle, UK)	100	89
El-Mofty et al (2008)	USA	2008	Unknown	32	Unknown	28/4	22	ISH	26	≥70	Clone unknown (Novacastra Labs Ltd, Newcastle, UK)	85	100
Evans et al (2011)	USA	2011	2000–2007	30	55	25/5	22	PCR and ISH	22	≥70	JC8 (Lab Vision, Fisher Scientific, Pittsburgh, PA, USA)	NA	NA
Farshadpour et al (2011)	The Netherlands	2011	1980–2004	32	Unknown	Unknown	14	ISH	14	≥70	Clone unknown (Neomarkers, Fremont, CA, USA)	93	100
Friedland et al (2012)	Australia	2011	1996–2008	20	Unknown	Unknown	19	PCR	19	Verbal definition	Clone unknown (Santa Cruz Biotechnology, Santa Cruz, CA, USA)	100	100
Gao et al (2013)	USA	2012	1997–2006	150	56	136/14	122	PCR	131	≥5 and <70	E6H4 (MTM Laboratories AG, Heidelberg, Germany)	93	100
Harkamp et al (2008)	The Netherlands	2008	1992–2001	81	58	59/22	33	PCR	37	≥5 and <70	E6H4 (Dako, Glostrup, Denmark)	86	98
Hoffmann et al (2012)	Germany	2012	2004–2009	20	Unknown	16/4	12	ISH	14	≥5 and <70	CINtecÒ (MTM Laboratories AG, Heidelberg, Germany)	79	83
Holzinger et al (2012)	Germany	2012	1990–2008	196	57	146/50	97	PCR	54	Verbal definition	E6H4 (MTM Laboratories AG, Heidelberg, Germany)	78	59
Hong et al (2010)	Australia	2010	1987–2006	195	59	159/36	83	PCR	65	Verbal definition	JC2 (Neomarkers, Fremont, CA, USA)	95	84
Junor et al (2012)	Scotland	2012	1999–2001 and 2003–2005	254	60	182/72	133	PCR	94	Verbal definition	E6H4 (MTM Laboratories, Heidelberg, Germany)	95	46
Kim et al (2007)	South Korea	2007	1995–2005	52	Unknown	Unknown	38	PCR	37	Verbal definition	Unknown	NA	NA
Klussmann et al (2009)	Germany	2009	1997–2005	60	60	47/13	29	PCR	33/57	≥5 and <70	16P04 (Neomarkers, Fremont, CA, USA)	NA	NA
Kuo et al (2008)	Taiwan	2008	1997–2005	92	51	79/13	69	PCR and ISH	49	≥5 and <70	JC8 (Neomarkers, Fremont, CA, USA)	NA	NA
Laco et al (2011)	Crech Republic	2010	2000–2009	22	60	13/9	18	PCR	22	Verbal definition	CINtec (MTM Laboratories AG, Heidelberg, Germany)	NA	NA
Lewis et al (2010)	USA	2010	1997–2008	239	55	211/28	144	ISH	187	≥5 and <70	Clone unknown (MTM Laboratories AG, Heidelberg, Germany)	74	90
Li et al (2007)	China	2007	1985–2004	49	58	37/12	9	PCR and ISH	11	Verbal definition	16p04 (Neomarkers, Fremont, CA, USA)	89	95

Table 1. (Continued)

Author (year)	Country	Year	Study years	n	Age (years)	M/F	HPV+	Analysis	P16+	P16 cutoff (%)	P16 antibody	Sensitivity of p16 (%)	Specificity of p16 (%)
Licitra et al (2006)	Italy	2006	1990–1999	90	58	69/21	17	PCR	32	Verbal definition	Clone unknown (Neomarkers, Fremont, CA, USA)	NA	NA
Lindquist et al (2012)	Sweden	2012	1970–2002	73	59	59/14	36	ISH	40	⩾5 and <70	Clone unknown (Pharmingen, San Diego, CA, USA)	73	60
Mellin Dahlstrand et al (2005)	Sweden	2005	1983–1999	51	63	39/12	25	PCR	27	⩾5 and <70	E6H4 (DakoCytomation A/S, Carpinteria, CA, USA)	74	79
Mills et al (2012)	USA	2012	Unknown	62	Unknown	Unknown	33	PCR and ISH	37	⩾5 and <70	E6H4, predilute, Tris (pH 9.0) (MTM Laboratories Inc., Westborough, MA, USA)	NA	NA
Nichols et al (2009)	USA	2008	Unknown	44	Unknown	35/9	27	PCR	29	Verbal definition	Clone unknown (MTM Laboratories, Heidelberg, Germany)	100	100
Ukpo et al (2011)	USA	2011	Unknown	211	56	188/23	153	ISH	148	Verbal definition	E6H4 (MTM, Laboratories Inc., Westborough, MA, USA)	98	90
Park et al (2012)	Korea	2011	2002–2007	93	62	80/13	53	PCR	46	Verbal definition	P2D11F11 (Novocastra Labs Ltd, Newcastle, UK)	100	85
Preuss et al (2008)	Germany	2008	1998–2005	106	57	77/29	30	PCR	61	Verbal definition	16p04 (Neomarkers, Fremont, CA, USA)	NA	NA
Quon et al (2013)	USA	2011	Unknown	48	Unknown	Unknown	36	PCR and ISH	35	Verbal definition	E6H4 (MTM Laboratories Inc., Westborough, MA, USA)	91	69
Reimers et al (2007)	Germany	2007	1997–2002	106	59	83/23	30	PCR	29	⩾5 and <70	16p04 (Neomarkers, Fremont, CA, USA)	86	86
Schache et al (2011b)	UK	2011	1988–2009	108	58	83/25	36	PCR and ISH	42	⩾70	CINtec (MTM Laboratories AG, Heidelberg, Germany)	NA	NA
Semrau et al (2012)	Germany	2012	2000–2008	52	56	42/10	15	PCR and ISH	17	⩾5 and <70	Clone unknown (Roche MTM Laboratories, Westborough, MA	NA	NA
Shi et al (2009)	Canada	2009	2003–2006	111	57	82/29	73	PCR and ISH	72	Verbal definition	CINtec (MTM Laboratories, Westborough, MA, USA)	NA	NA
Thavaraj et al (2011)	UK	2011	Unknown	142	58	108/34	100	PCR and ISH	90	⩾70	CINtec (MTM Laboratories Westborough, MA, USA)	97	75
Ukpo et al (2012)	USA	2012	1996–2007	154	56	133/21	89	ISH	104	Verbal definition	E6H4 (MTM Laboratories Inc., Westborough, MA, USA)	NA	NA
Weinberger et al (2010)	USA	2010	1980–1999	140	60	106/34	58	ISH	25	Verbal definition	JC8 (Abcam Corporation, Cambridge MA, USA)	100	57
Weiss et al (2012)	Germany	2012	Unknown	61	Unknown	Unknown	36	PCR	30	⩾5 and <70	Unknown	NA	NA
Zhao et al (2012)	USA	2012	2002–2006	38	Unknown	Unknown	14	ISH	22	Verbal definition	MAB4133 (Chemicon International Company/Millipore Corporation, Temecula, CA, USA)	NA	NA

Abbreviations: HPV = human papillomavirus; ISH = *in situ* hybridisation; NA = not applicable; PCR = polymerase chain reaction.

Table 2. Patient characteristics	
Demographics	**Total ($n = 3926$)**
Age, median (range)	58 (20–93)
Sex[a], $n = 3625$	
Male	2921
Female	704
Country, $n = 3926$	
Europe	1446
Australia	215
USA	1759
Asia	506
Ethinicity, $n = 3926$	
Caucasian	1568
Asian	269
Mixed	428
Unspecified	1661
Tumour origin, $n = 3926$	
Base of tongue	414
Palatine tonsils	1420
Unspecified/oropharynx	2092
Definition of p16 overexpression, $n = 3926$	
Staining equals or exceeds 70% of nuclei and cytoplasm	764
Staining between 5 and 69% of nuclei and cytoplasm	1684
Verbal definition	1478

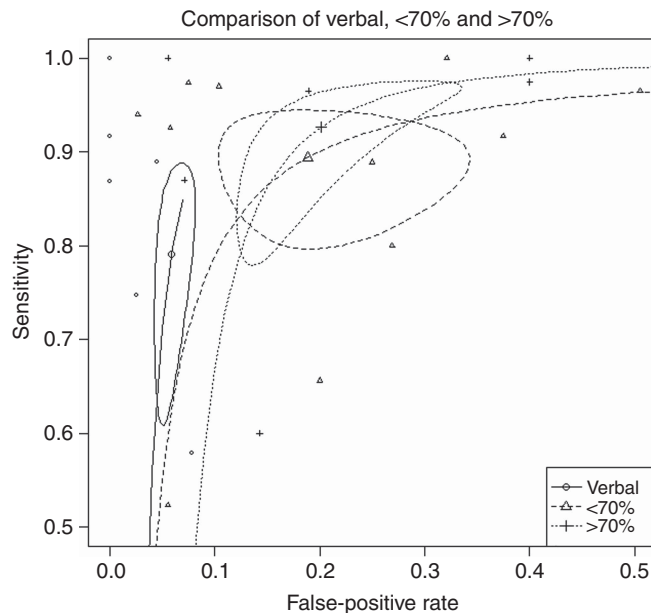[a]$N = 301$ subject's sex is unknown.

Figure 2. Hierarchical summary receiver-operator curve (HSROC) of the studies from Table 1. The studies have been divided into three groups based on their definition of p16 staining: a verbal group, a <70% group, and a >70% group, including 95% CIs for the summary point. The verbal group has a lower false discovery rate, while the >70% group had a greater overall sensitivity and a smaller 95% CI.

no statistically significant difference between groups of p16 definition correlated to HPV, which may be because of the great heterogeneity among studies, including different p16 antibodies. In addition, ISH and PCR methods vary from centre to centre, leading to a loss of statistical power to detect differences. The explanation might also be that all p16 groups are equally correlated to HPV status; thus, the level of p16 staining is less important and the status of positivity or negativity is evident for a given staining, that is, most p16-positive tumours are above 70% when positive. Histopathologic grade and morphology was insufficiently reported and an agreement on a grading scheme applicable to OPSCC and consensus on reporting data is important for future research. As to p16 antibodies, an FDA-approved recommendation might be profitable to uniform research methods. It is widely assumed that HPV-related oropharyngeal cancers are poorly differentiated based on the immature appearance of the tumour cells, but in fact they are commonly highly differentiated as they emulate the specialised epithelium of the tonsillar crypts (Westra, 2009). Further data for analysis on this matter might question the challenge of interpreting p16-IHC in mixed and keratinising SSCs. In addition, it should be considered if carcinoma *in situ* should be included in future similar studies.

In future studies applying p16-IHC and HPV diagnostics, the real value of IHC must be questioned once the site of the tumour is known (oropharynx) and the morphology is recognised (non-keratinising); the chance of a non-keratinising OPSCC being HPV positive is still not known.

Previous data report a prevalence of HPV in OPSCC of 51%, which is similar to our results (O'Rorke *et al*, 2012). Regardless if studies used PCR, ISH, or both, similar results were achieved.

Oropharyngeal squamous cell carcinomas are characterised by a heterogeneous clinical and molecular profile (Huang *et al*, 2002; Shah and Patel, 2003; Bosch *et al*, 2004; De Vita *et al*, 2008) and

have interestingly proven to have a better prognostic outcome in cases with p16 overexpression (Lewis *et al*, 2010; Ang *et al*, 2010a). P16-IHC is, however, a diagnostic method causing much debate, and concerns have been raised: p16 overexpression might be associated with functional pRb disturbances irrelevant for the HPV infection (Marur *et al*, 2010). High-risk human papillomavirus-infected OPSCCs have not necessarily lost the 9p21 allele encoding p16 (Braakhuis *et al*, 2004), and p16-IHC has been reported 100% sensitive but 79% specific as to carcinomas with HPV infection (Smeets *et al*, 2007). P16-IHC is performed on just one slide of tumour tissue and staining might vary allowing false-negative results explaining a lower specificity. Lately, cutoff values above 70 or 75% have proven to be of wider use (Ang *et al*, 2010a; Evans *et al*, 2011; Schache *et al*, 2011a) as compared with, e.g., values >10% as a 'validated' definition of p16 overexpression. In a retrospective study based on material from The Danish Society for Head and Neck Oncology (DAHANCA), the cutoff value was changed in a Letter to the Editor after publication from >10 to >70% (Lassen and Overgaard, 2012).

In conclusion, substantial differences exist in the definition of p16 overexpression and means of HPV diagnostics between studies. To achieve the highest correlation between p16-IHC and HPV results, we advise clinicians and researchers to define p16 overexpression as >70% staining of tumour cells. Future research in this field should report on p16 and HPV results, allowing a better understanding of the association between the two.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

CB concepted the idea of the study. CGL and MG searched the scientific literature, extracted data, and led the writing. LK, CGL,

and DHJ performed statistics. All authors provided conceptual input, interpreted the findings, and contributed in significant ways to the final article.

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