The evolving influence of Human Papillomavirus on Oropharyngeal Squamous Cell Carcinoma in the UK: a cross-sectional study 2002-2011

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Key Words

Head and Neck Cancer, Oropharyngeal Cancer, Human papillomavirus, HPV

Abstract

Objectives

In much of the developed world, substantial increases in the incidence of oropharyngeal squamous cell carcinoma (OPSCC) have been observed and attributed to Human Papillomavirus (HPV). This study aimed to determine the proportion of OPSCC associated with HPV in the UK population between 2002 and 2011.

Design

This pan-UK, cross-sectional study used archival tumour tissue. HPV status was determined using three well-validated commercial tests, performed in accredited laboratories (p16 immunohistochemistry, HPV DNA in-situ hybridisation, and multiplex HPV PCR). Incidence data were obtained from UK Cancer registries.

Setting

Archival tissue blocks from patients diagnosed with OPSCC between 2002 and 2011, were collected from eleven centres across the four constituent countries of the UK. An equal number of cases was requested for each year.

Participants

Archival tissue blocks were obtained from 1602 patients with confirmed OPSCC; 1529 were tested for HPV; and valid data were obtained for 1474 cases. The mean age of analysed patients was 59.3 years, 75.0% of patients were male, and most cases (57.9%) were from the tonsil.

Main outcome measures

The study determined the overall proportion of OPSCC caused by HPV. HPV prevalence was also assessed by gender, age and anatomical subsite. Change over time was calculated. Overall incidence

rates for OPSCC in the UK were calculated, and the proportion of OPSCC attributable to HPV was estimated.

Results

The proportion of OPSCC caused by HPV between 2002 and 2011 was 51.8% (95% CI: 49.3, 54.4). There was no change in the proportion of HPV-attributable cases over time (unadjusted risk ratio: 1.00 (95% CI: 0.99, 1.02). However, over the same period in the UK, the incidence of OPSCC approximately doubled (age standardised rate (ASR) 2002: 2.1 (95% CI: 1.9, 2.2); 2011: 4.1 (95% CI: 4.0, 4.3)).

Conclusions

The incidence of OPSCC in the United Kingdom increased between 2002 and 2011 with most cases occurring in men. Approximately half of all OPSCC cases were HPV positive, but there was no increase in the proportion of HPV-attributable cases. Therefore the increasing incidence of OPSCC is not solely due to HPV, and the reasons for the parallel increase in HPV-negative disease warrant investigation.

Introduction

The developed world has experienced a dramatic rise in the incidence of oropharyngeal squamous cell carcinoma (OPSCC).[1-3] In England between 1995 and 2011, the Age-Standardised incidence Rate (ASR) for OPSCC approximately tripled in men (from 2.0 to 5.8) and doubled in women (from 0.8 to 1.7).[4] The association between tobacco and alcohol consumption and OPSCC is well established,[5] but sexual behaviour is also a risk factor, with lifetime number of oral sex partners recognised as the behavioural measure most strongly associated with the development of OPSCC.[6] Changes in sexual behaviour appear likely to underlie the increasing proportion of OPSCC attributable to oncogenic Human papillomavirus (HPV).[2 5 7] Several studies in Europe and North America have confirmed sharp rises in HPV-induced OPSCC incidence, although the exact proportion of HPV positive tumours within the total disease burden varies considerably by geographical region.[8-12]

HPV is best-known as the cause of cervical cancer in women. However OPSCC predominantly affects men; in England in 2011, the ratio of male:female cases of OPSCC was 3.4:1.[4] Epidemiological studies in the USA showed that the typical HPV-positive OPSCC patient is a middle-aged, non-smoking, white man of higher socioeconomic status, with a history of multiple sexual partners and/or orogenital sexual partners.[13]

In the UK, the proportion of OPSCC attributable to HPV has been assessed in several single centre studies, but these were small, used diverse methods and had limited geographical coverage.[14-16] The current study aimed to assess the proportion of OPSCC attributable to HPV infection using robust, standardised methods in a large contemporary sample (2002-2011 inclusive), covering the entire United Kingdom. These data are required to facilitate health economic analyses, and to inform evidence-based policy-making with regard to prophylactic vaccination to prevent HPV infection in boys, as recently implemented in Australia.[17-19]

Methods

Case selection

The study received Research Ethics Committee approval (REC 11/NQ/0452). OPSCC was defined as base of tongue (C01), soft palate and uvula (C05.1 & C05.2), tonsil (C09) and oropharynx-not-otherwise-specified (C10.9). OPSCC cases diagnosed between 2002 and 2011 (inclusive) were collected from 11 recruiting centres, which were distributed across the UK to ensure results were not distorted by effects in one area or centre (Belfast, Bristol, Cardiff, Coventry, Edinburgh, Liverpool, London, Manchester, Newcastle, Poole and Southampton). The overall target sample-size (1710) was sufficient to allow comparison of prevalence between years with 7.5% precision. The number of samples per centre was determined pragmatically, based on the number of cases seen annually at each centre. To avoid selection bias, the first 17 cases per year (11 cases for Coventry and Bristol) with available formalin-fixed paraffin embedded (FFPE) tumour blocks were included (irrespective of the definitive treatment modality employed). A representative FFPE block, either from diagnostic or resection specimen, was selected. Gender, age at diagnosis, year of diagnosis and histological diagnosis, including anatomical subsite classification, were recorded.

HPV testing

Sections of each FFPE block were taken for DNA analysis. To prevent DNA contamination, the microtome was thoroughly cleaned between specimens and a new blade was used for each block. Tissue microarrays (TMA) were constructed for p16 immunohistochemistry and high risk HPV DNA in-situ hybridisation testing as previously described.[20] Following construction, haematoxylin and eosin-stained sections of the TMAs were analysed to confirm accuracy of sampling. Samples were only considered as adequate for analysis if all three TMA cores included tumour.

For PCR, DNA was extracted from 2 x 10 µm whole sections of original FFPE blocks by digestion for 16 hours in Tris 50mM / EDTA 1mM / Tween 0.5% with 1mg/mL Proteinase K at 56°C, followed by heat inactivation (100°C for 5 mins) and centrifugation. Extracted DNA was tested for the presence of HPV DNA using the Optiplex HPV Genotyping Kit (Diamex GmbH, Heidelberg, Germany) according to the manufacturer's instruction. This assay uses luminex technology to detect 24 common HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82). PCRs were performed using the Qiagen Multiplex PCR Kit (Qiagen GmbH, Hilden, Germany). The assay includes primers for amplification of the human ß-globin gene to confirm sample adequacy. Appropriate controls were included for sectioning (blank paraffin block), DNA extraction (reagent blanks), and HPV testing (HPV-positive Caski cell line DNA as a positive control and water as a negative control).

p16 immunohistochemistry (IHC) was carried out, as a surrogate marker of HPV oncogene expression,[21] using a proprietary kit (CINtec p16 Histology, Ventana Medical Systems, Mountain View, USA) on a Ventana Benchmark Autostainer. p16 IHC was scored as positive if there was strong and diffuse nuclear and cytoplasmic staining present in greater than 70% of the malignant cells.[21] All other patterns were scored as negative.

High-risk HPV DNA ISH was carried out using proprietary reagents (Inform HPV III Family 16 Probe (B), Ventana Medical Systems, Mountain View, USA) on a Ventana Benchmark Autostainer. The Inform HPV III Family 16 Probe (B) detects high-risk HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66. The high-risk HPV ISH test was scored as positive if there was any blue reaction product that co-localised with the nuclei of malignant cells.[22] Focal specific staining of only part of the tumour section was regarded as positive. Diffuse staining of tumour and stromal tissues, considered to represent non-specific chromogen precipitate, was scored as negative. Pale staining limited to the nucleoli of cells and staining of occasional leucocytes and stromal cells were also disregarded, in line with the manufacturer's instructions.

p16 IHC and high-risk HPV DNA ISH were assessed by a panel of experienced head and neck pathologists. To quality assure this process, the panel undertook a calibration exercise on a training set of OPSCC cases (whole sections and TMAs) prior to scoring the study material.

Classification of HPV status

All cases were tested using all three assays, and were classified as HPV-positive if they showed evidence of both HPV gene expression (indicated by p16 IHC) and HPV DNA (indicated by ISH and/or PCR). A diagnostic algorithm utilising p16 IHC, HR HPV DNA ISH and PCR in a stepwise fashion was applied.[22 23] The results of individual HPV diagnostic tests, and clinically-relevant combinations of tests are also reported.[24]

Incidence Data

Contemporaneous data on cancer incidence were obtained from Office of National Statistics (ONS) in England, NHS National Services Scotland, Northern Ireland Cancer Registry and Welsh Cancer Intelligence & Surveillance Unit. Age standardised rates (ASR) were calculated, for the following groups: oropharyngeal cancers (comprised of C01, base of tongue; C05.1, soft palate; C05.2, uvula; C09, tonsil; C10.9, oropharynx not-otherwise-specified). ASR were also calculated for C32 (larynx), as a representative head and neck subsite not thought to be associated with HPV infection.[23] ASR were calculated using the updated 2013 European Standard Population, and mid-2012 UK population estimates.[25]

Statistical analysis

The characteristics of included cases (gender, age at diagnosis, oropharyngeal subsite, year of diagnosis and study centre) were described using frequencies/percentages or means/standard deviations as appropriate. These characteristics were compared with excluded cases, and also with UK Cancer Registry data using t-tests or chi-squared tests as appropriate. The proportion of HPV-associated cases was calculated for the whole sample, then for each subset (with 95% confidence intervals). Age was the only continuous variable, and was categorised into five groups. The proportion of cases positive by p16 IHC, high-risk HPV DNA ISH and HPV PCR was also calculated to allow comparison with studies reporting these endpoints, as was the prevalence of HPV types among cancers caused by a single HPV type. Trends in the proportion of HPV-associated cancers over time were assessed using Poisson regression with robust error variance.[26] This approach was used as odds ratios (obtained from logistic regression) are poor approximations of risk ratios if the outcome prevalence is high. Models were fitted before and after adjusting for the sample characteristics. Finally, gender-specific ASR were calculated from the UK cancer registry data, and Annual Percentage Change (APC) were obtained by fitting linear regression models with logged ASR as the outcomes. HPV-positive proportions determined in the current study were applied to the incidence data to estimate the burden of oropharyngeal cancers caused by HPV over the period. All analyses were undertaken in Stata 14.0 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX, USA).

Results

Case characteristics

Archival OPSCC tissue blocks were obtained from 11 UK centres. Figure 1 illustrates the application of study inclusion criteria and the HPV diagnostic testing algorithm. Valid results were obtained for 1474 cases. Sample characteristics (gender, age at diagnosis, oropharyngeal subsite, year of diagnosis and study centre) are shown in Table 1. The mean age of patients was 59.3 years, 75.0% of

patients were male, and the majority of cases (57.9%) were from the tonsil. Invalid results were obtained for 55 patients and these were excluded from the analysis. The age, gender and subsite distribution were similar for the included and excluded samples, and excluded samples were evenly distributed across the study period. The reasons for exclusion were either absence of tumour in the TMA cores or loss of TMA cores during processing for staining.

The age and gender distributions were compared between OPSCC cases included in the current study, and those reported by UK Cancer Registries for the same period (Supplementary Table 1). The study sample included 8.3% of the 17739 OPSCC diagnosed 2002-2011. The gender balance between the current study (75% Male) and the UK OPSCC population (73.5% Male) was similar. There was a higher proportion of younger patients (45-54.9 years) in the study sample (30.4%) than in the OPSCC population overall (25.7%) (p=0.001); however the age distribution for cases in the two halves of the study period was similar (2002-2006 vs. 2007-2011, p=0.5) (Supplementary Table 2).

Prevalence of HPV

The prevalence of HPV infection in OPSCC was 51.8% (95% CI: 49.3, 54.4). The prevalence of HPV infection within specific subgroups is shown in Table 2. The proportion of HPV-positive cases was higher in men than women (54.3% vs 44.4%), and decreased with increasing age (69.2% in patients aged less than 44 years vs 37.2% in patients aged over 75 years). The mean age of patients with HPV-positive disease was 57.4 years compared to 61.4 years for HPV-negative cases (p<0.001). The prevalence of HPV infection varied depending on the tumour site, with the tonsil subsite showing the highest prevalence (61.8%), and soft palate/uvula showing the lowest (9.1%). Prevalence also varied across study centres, from 67.5% (95%CI 59.7, 74.4) in Liverpool to 35.4% (95%CI 27.0, 44.8) in Belfast. The variation in the overall proportion of samples defined as positive by each test (53.7% p16 positive, 45.0% ISH positive, 66.6% PCR positive) is consistent with published literature, and reflects the established sensitivities and specificities of the assays.[14-16]

There appeared to be little variation in prevalence across the 10 year study period (Figure 2), either with HPV-positives defined using the stepwise algorithm, or for individual tests. Statistical models were used to assess change in the proportion of HPV-positive cases per year; the unadjusted risk ratio was 1.00 (95% CI: 0.99, 1.02) for HPV infection for each year compared to the previous year (p for linear trend = 0.6), and the risk ratio adjusted for gender, age at diagnosis, anatomical subsite and study centre was 1.00 (95% CI: 0.98, 1.03) (p value for linear trend=0.7). This confirmed the absence of change in the proportion of HPV-positive samples over time.

Type-specific HPV prevalence

Among the 764 HPV-positive OPSCC, the Optiplex HPV Genotyping Kit identified a specific high-risk HPV type in 732 cases. In 710 (97%) of these cases a single HPV type was present; the other 22 samples contained DNA of more than one HPV type. Among cases with a single HPV type detected, HPV16 was present in 684/710 cases (96.3%, 95%CI 94.7, 97.6) and HPV 18 in 11/710 cases (1.5%, 95%CI 0.8, 2.8) (Supplementary table 3). Among cases classified as HPV-positive, HPV 16 and/or 18 were identified in 714/764 (93.5%, 95%CI 91.5, 95.1). HPV33 was detected in 20 cases (2.6%, 95%CI 1.6, 4.0); in 9 of these cases, HPV16 was also present.

Oropharyngeal and laryngeal cancer incidence 2002-11 and estimated HPV-associated disease burden

In the UK, between 2002 and 2011, the incidence of OPSCC more than doubled (APC 8.1%), while the incidence of laryngeal cancer increased only marginally (APC 1.0%) (Figure 3). The majority of OPSCC occurred in men (2011 ASR: 6.3 male vs 2.1 female) and incidence increased most rapidly in men (2002-2012 APC male 8.4% vs. female 7.5%). Laryngeal cancers were also more common in men than women (2011 ASR:7.1 vs 1.3) and also increased most rapidly in men (APC male 1.2% vs. female

0.6%). To estimate the burden of oropharyngeal cancers caused by HPV over this period, the proportions determined in the current study were applied to the incidence data (Figure 4). Figure 4 highlights an increasing incidence of both HPV-positive and negative OPSCC, especially in men.

Discussion

This first UK-wide study of the prevalence of HPV in OPSCC showed that 51.8% (95% CI: 49.3, 54.4) of cases of OPSCC diagnosed between 2002 and 2011 were HPV-positive. Over the same period, the incidence of OPSCC in the UK approximately doubled (ASR 2002: 2.1, ASR 2011: 4.1). The relative proportions of HPV-positive and negative OPSCC remained stable over time. These data demonstrate a parallel rise in HPV-positive and negative disease incidence that has not previously been reported. This shows that the increasing incidence of OPSCC cannot be explained solely by an increase in HPV-associated disease.

The strengths of the study include: substantial sample size; broad geographical representation; rigorous and systematic case selection; and, use of well-validated commercial tests to identify HPV infection. The results are likely to reflect national trends, although there is potential for variation in OPSCC incidence and in HPV prevalence between some local geographical areas. To assess potential bias in case selection, the records supplied by each centre were formally reviewed. This showed that FFPE blocks from only nine HPV-positive patients were unavailable due to use in other UK clinical studies. A higher proportion of younger patients (45-54.9 yrs) relative to the UK OPSCC population was included in the study. Given the younger mean age for HPV-positive patients, this would be more likely to result in overestimation of the proportion of HPV-associated disease, rather than underestimation. The HPV testing regime included three independent, well-validated, commercial tests (IHC, ISH and PCR), performed in independent laboratories. The three tests showed highly similar trends in HPV prevalence (Figure 2). The analysis of data pertaining to behavioural factors, such as smoking and sexual history, could potentially have allowed further interpretation of our

results, however due to the retrospective nature of sample and data collection these data could not be reliably obtained.

Substantial variation has been reported in the proportion of OPSCC attributable to HPV, between countries and time periods.[11] This likely reflects variation in multiple factors including sexual behaviour and rates of genital HPV infection, as well as tobacco and alcohol consumption. Previous small, single centre studies from the UK reported HPV prevalence rates in OPSCC of 37.5% (95% CI: 28-48%), 42.7% (95% CI: 36-50%), and 55% (95% CI: 45-66%).[14-16] The current study is consistent with these, but is based on a much larger sample with broader geographical representation, including centres in all four countries of the UK. We observed a consistent proportion of HPV-positive OPSCC over time from 2002 to 2011, against a background of increasing incidence. This contrasts with some previous data, where an increased proportion of HPV-positive OPSCC was associated with increasing incidence of OPSCC overall.[9] Recent data from North America[11] and Stockholm,[27] however, suggest that plateaus in the proportion of HPV-positive OPSCC and HPV-positive tonsil SCC respectively, have been observed from the year 2000 onwards.

The absence of change in the proportion of HPV-associated disease, despite a continued rise in incidence of OPSCC, is difficult to explain. It implies that HPV-negative OPSCC, traditionally associated with smoking, is also increasing in incidence. This contrasts with the stable incidence of other smoking-related head and neck malignancies, such as laryngeal cancer[28] and suggests that another risk factor, in addition to HPV and smoking, may contribute to the increased overall incidence of OPSCC. Prior tonsillectomy appears to reduce risk of tonsillar carcinoma,[29 30] but while the current UK tonsillectomy rate is approximately 75% lower than in the 1950s,[31] the absence of a disproportionate increase specifically in cancers of the tonsil subsite, in our results or published data,[28] suggests this is not a major contributory factor to increasing OPSCC.

It is likely that prophylactic vaccination will prevent HPV-positive OPSCC,[32] and the UK Joint Committee on Vaccination and Immunisation are considering extending prophylactic HPV

vaccination to include boys, as well as girls.[32 33] Our data substantially expand the evidence base available to inform this decision. The current bivalent and quadrivalent HPV vaccines protect against infection with the oncogenic HPV types 16 and 18; these types were present in 714/764 cases (93.5%, 95%CI 91.5, 95.1) of HPV-positive OPSCC. Our data suggest that of the 1781 OPSCC diagnosed in men in 2011, approximately 926 were HPV-positive, and 866 were associated with HPV types included in current vaccines.

The reasons for the rise in HPV-negative OPSCC incidence, demonstrated by our data, remain unclear. In an analysis of Dutch HNSCC incidence, van Monsjou et al.[34] noted that behavioural changes in the post-World War II generation included reduced smoking rates coupled to significant rises in alcohol consumption; and suggest that excessive alcohol intake alone may be a more critical risk factor for OPSCC than smoking. However, it seems incongruous that alcohol should exert a differential oncogenic effect on oropharyngeal mucosa compared to that of the oral tongue, floor of mouth, or hypopharynx. Alternative explanations for the observed increase in HPV-negative OPSCC could include an unidentified infectious agent (possibly creating a tumour promoting microenvironment, through increased inflammation or proliferation), although the presence of novel directly carcinogenic viruses appears unlikely given the absence of novel viral sequences in mRNA sequencing analyses.[35] The parallel increase in both HPV-positive and HPV-negative tumours should be of concern to those involved in the clinical management of OPSCC, and to public health officials charged with developing strategies to reduce incidence. Further research is necessary to explain the increase in HPV negative OPSCC and to delineate the natural history of both HPV-positive and negative OPSCC; such research will inform both prevention and treatment of these increasingly common cancers.

WHAT IS KNOWN

The incidence of Oropharyngeal Squamous Cell Carcinoma (OPSCC) has increased rapidly over recent decades. These trends were reported primarily in developed nations and have been attributed to increased incidence of HPV-associated cancers.

WHAT THIS RESEARCH ADDS

We defined the proportion of OPSCC caused by HPV in the UK between 2002 and 2011 as 51.8% (95% CI: 49.3, 54.4). Surprisingly, there was no change in the proportion of HPV-attributable cases over this time. Over the same period, the UK incidence of OPSCC approximately doubled, but the incidence of laryngeal cancers (mostly associated with smoking) remained stable. The data show that HPV-positive OPSCC present an increasing disease burden, but also suggest the presence of an unidentified risk factor associated with HPV-negative disease.

Contributorship statement

TJ, ME, MR and NP conceived and designed the original protocol. All authors were involved in amending the protocol. All authors were involved in identifying and submitting archival tumour blocks. NP, MR, DR, and KC performed the laboratory analyses and MR, HMo, MP, GT, DMcC and JJ undertook FFPE-based staining interpretation. AS, NP and SL were responsible for data analysis. AS and NP wrote the first draft of the manuscript. All authors contributed to subsequent and final drafts. TJ is the guarantor of the paper.

Acknowledgements

We would like to acknowledge the assistance of the staff of the National Cancer Registries, especially Jaroslaw Lang (Information Services Division, NHS National Services Scotland), Deirdre Fitzpatrick (Northern Ireland Cancer Registry) and Ceri White (Welsh Cancer Intelligence & Surveillance Unit).

Funding

This independent research was supported by a research grant from GlaxoSmithKline Ltd (Middlesex, United Kingdom). The Northern Ireland Biobank is funded by the Health and Social Research Development Division of the Public Health Agency. Study design, execution, interpretation and manuscript preparation was undertaken by authorship as detailed without external influence.

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Table 1. Characteristics of included/excluded cases

Variable		Included in study (n=1474)		Excluded from study (n=55)		P value for difference[a]
		Mean	**Standard deviation**	**Mean**	**Standard deviation**	
Age at diagnosis		59.3 years	10.7 years	57.3 years	8.6 years	0.2
		Frequency	**Percentage**	**Frequency**	**Percentage**	
Gender	M	1150	75.0	32	58.2	0.01
	F	369	25.0	23	41.8	
Oropharyngeal Subsite	Tonsil	854	57.9	26	47.3	0.1
	Base of Tongue	362	24.6	20	36.4	
	Soft palate	88	6.0	5	9.1	
	Oropharynx NOS[b]	170	11.5	4	7.3	
Year of diagnosis	2002	118	8.0	6	10.9	0.7
	2003	133	9.0	4	7.3	
	2004	135	9.2	9	16.4	
	2005	148	10.0	5	9.1	
	2006	156	10.6	5	9.1	
	2007	159	10.8	7	12.7	
	2008	168	11.4	3	5.5	
	2009	149	10.1	4	7.3	
	2010	145	9.8	6	10.9	
	2011	163	11.1	6	10.9	
Study Centre	Belfast[c]	113	7.7	2	3.6	<0.001
	Bristol	65	4.4	0	0	
	Cardiff[d]	149	10.1	4	7.3	
	Coventry	92	6.2	7	12.7	
	Edinburgh[e]	108	7.3	27	49.1	
	Liverpool	157	10.7	4	7.3	
	London[f]	158	10.7	4	7.3	
	Manchester[g]	166	11.3	0	0	
	Newcastle	170	11.5	0	0	
	Poole	116	7.9	7	12.7	
	Southampton	180	12.2	0	0	

[a]p value for comparison between those included/excluded from the study, t-test for mean(SD), chi-squared test for percentages.

[b]NOS – Not Otherwise Specified.

[c]Belfast – samples were accessed via The Northern Ireland Biobank from the Belfast Health and Social Care Trust archives which serves the Royal Victoria Hospital and the Belfast City Hospital.

[d]Cardiff – samples were collected via Velindre Cancer Centre, which serves all of South East Wales.
[e]Edinburgh – samples were collected via the East of Scotland Cancer Centre which serves Edinburgh and surrounding areas, Dumfries and Galloway, Fife and the Scottish Borders.
[f]London – samples were collected via Royal Marsden Hospital, which serves South West and West London, with additional referrals from Sussex and Kent.
[g]Manchester – samples were collected via the Christie NHS Foundation Trust, which serves Greater Manchester and parts of Cheshire.

Table 2. Associations between characteristics and HPV status

		HPV Prevalence (%)[a]	95% CI
All samples		51.8	49.3, 54.4
Gender	Male	54.3	51.3, 57.2
	Female	44.4	39.4, 49.6
Age at diagnosis	≤ 44.9 years	69.2	59.6, 77.5
	45-54.9 years	61.4	56.8, 65.8
	55-64.9 years	48.9	44.6, 53.3
	65-74.9 years	42.3	36.7, 48.1
	≥ 75 years	37.2	29.2, 46.0
Oropharyngeal Subsite	Tonsil	61.8	58.5, 65.0
	Base of Tongue	49.4	44.3, 54.7
	Soft palate/Uvula	9.1	4.5, 17.3
	Oropharynx NOS[b]	28.8	22.5, 36.2
Year of diagnosis	2002	50.0	40.9, 59.1
	2003	48.1	39.7, 56.7
	2004	52.6	44.1, 61.0
	2005	54.1	45.9, 62.0
	2006	47.4	39.6, 55.4
	2007	55.4	37.0, 52.5
	2008	54.2	46.5, 61.6
	2009	53.7	45.6, 61.6
	2010	53.1	44.9, 61.2
	2011	49.1	41.4, 56.8
Study Centre	Belfast	35.4	27.0, 44.8
	Bristol	43.1	31.4, 55.6
	Cardiff	62.4	54.3, 69.9
	Coventry	41.3	31.6, 51.8
	Edinburgh	45.4	36.1, 55.0
	Liverpool	67.5	59.7, 74.4
	London	65.2	57.4, 72.3
	Manchester	51.2	43.6, 58.8
	Newcastle	44.1	36.8, 51.7
	Poole	52.6	43.4, 61.6
	Southampton	47.8	40.5, 55.1

[a]HPV prevalence defined according to tier-wise algorithm.

[b]NOS – Not Otherwise Specified.

Supplementary Table 1. Age and gender comparison between the current study and the UK incidence data (2002-2011)

		Current study (n=1474)[a]		UK incidence data (n=17738)[a]		P value for difference[b]
		Frequency	%	Frequency	%	
Age at diagnosis	≤ 44.9 years	104	7.1	1267	7.1	0.001
	45-54.9 years	448	30.4	4561	25.7	
	55-64.9 years	507	34.4	6417	36.2	
	65-74.9 years	286	19.4	3562	20.1	
	≥ 75 years	129	8.8	1931	10.9	
Gender	M	1105	75.0	13038	73.5	0.2
	F	369	25.0	4700	26.5	

[a]The 1474 cases included in the current study are present within the 17738 incident cases (i.e the groups are not mutually exclusive).

[b]p value for comparison between those in the current study and the UK incidence data, t-test for mean (SD), chi-squared test for percentages.

Supplementary Table 2. Comparison of age distribution in cases from 2002-2006 vs. 2007-2011

Age at diagnosis	2002-2006 (n=690)		2007-2011 (n=784)		P value for difference[a]
	n	%	n	%	
≤ 44.9	46	6.7	58	7.4	0.5
45-54.9	223	32.3	225	28.7	
55-64.9	230	33.3	277	35.3	
65-74.9	127	18.4	159	20.3	
≥ 75	64	9.3	65	8.3	

[a]p value from chi-squared test.

Supplementary Table 3. Type-specific HPV prevalence among single infected HPV-positive tumours

HPV type	Frequency	% [a]	95% confidence interval
HPV16	684	96.3	94.7, 97.6
HPV18	11	1.5	0.8, 2.8
HPV33	8	1.1	0.5, 2.2
HPV35	1	0.1	0.004, 0.8
HPV59	5	0.7	0.2, 1.6
HPV73	1	0.1	0.004, 0.8

[a] denominator = 710 samples defined as single infected HPV-positive using the diagnostic algorithm and with valid PCR typing results.

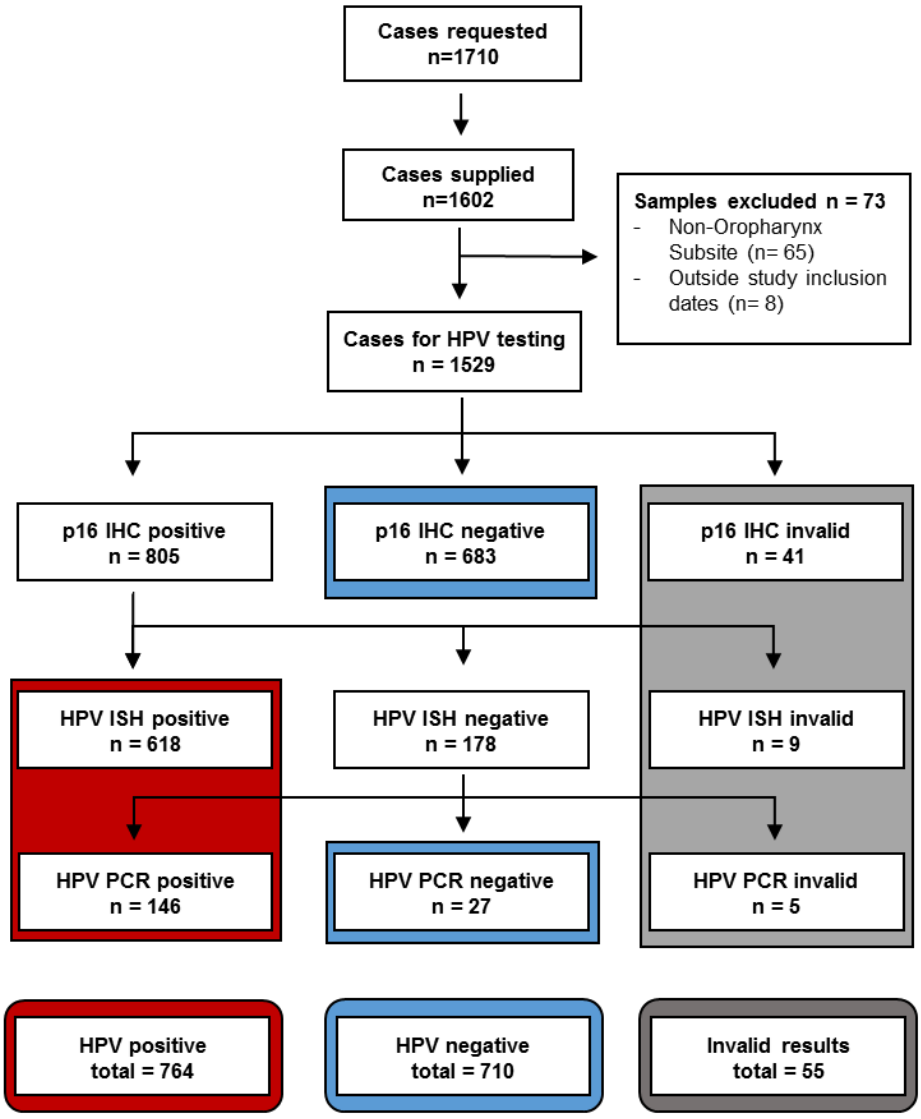

Figure 1. Study schema
The HPV testing algorithm was applied in three tiers. 1474 of 1529 samples were successfully classified as HPV positive (red boxes) or negative (blue boxes). 55 samples gave invalid data (grey boxes).

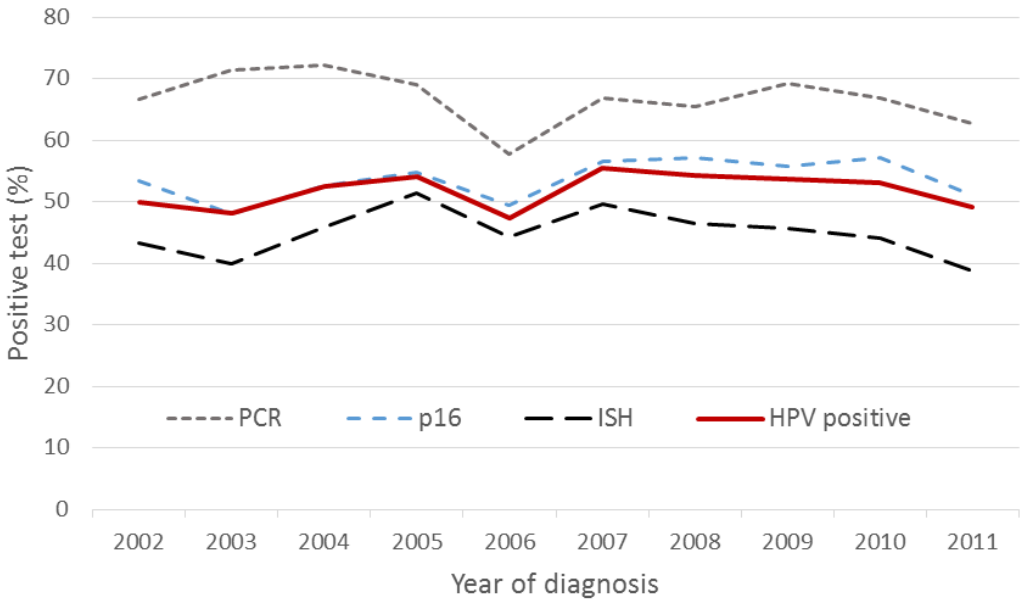

Figure 2. Proportion of OPSCC testing HPV positive over time, by individual test and algorithm

The red line represents the proportion of samples classified as HPV positive using the algorithm shown in Figure 1. Other lines show the results of individual tests.

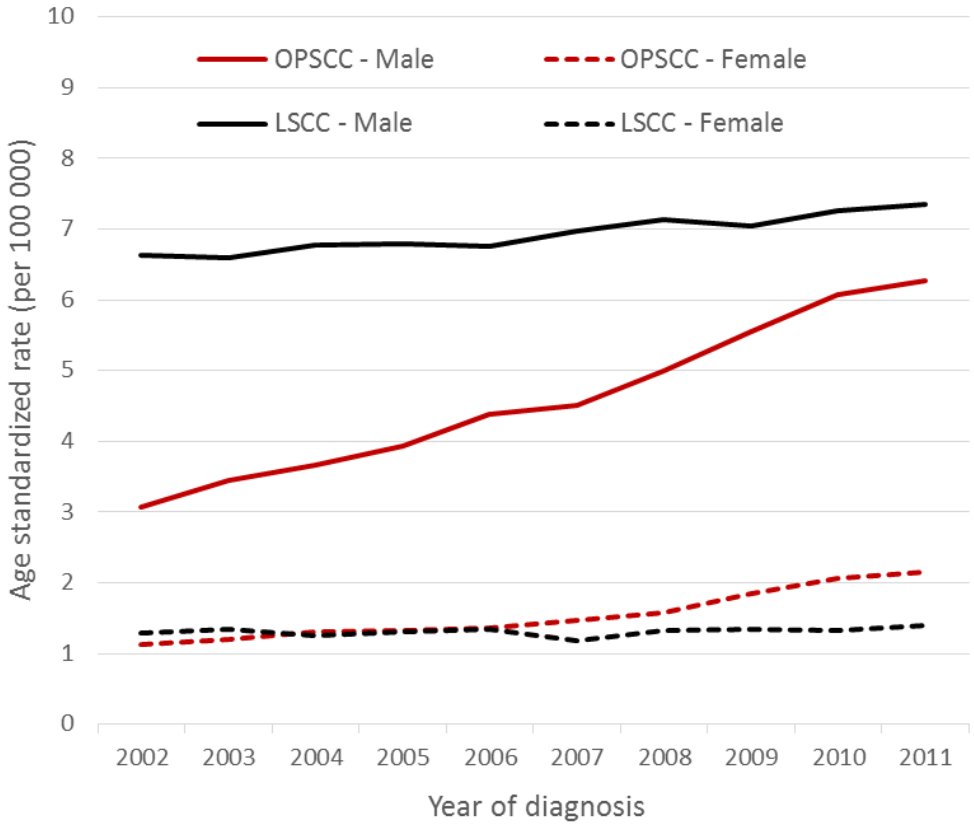

Figure 3. Incidence of SSC of the oropharynx and larynx (UK, 2002-2011)
OPSCC indicates Oropharyngeal Squamous Cell Carcinoma; LSCC indicates Laryngeal Squamous Cell Carcinoma

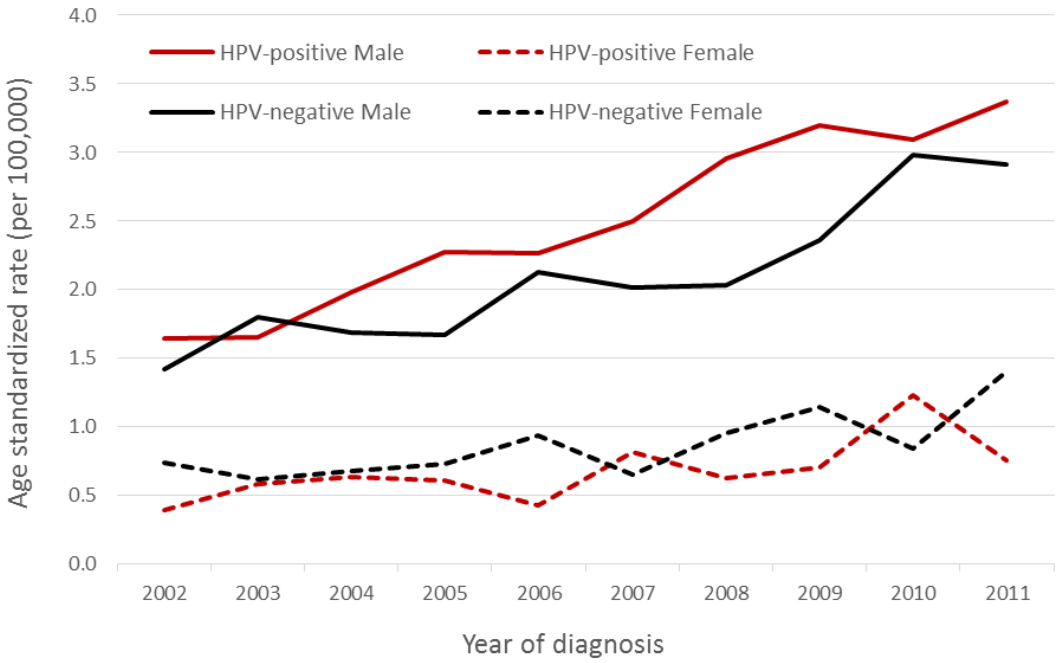

Figure 4. Estimated incidence of OPSCC by HPV status (UK, 2002-2011)
For each year, the gender-specific proportion of HPV positive samples was multiplied by the gender-specific incidence to estimate ASR for both HPV positive and negative OPSCC.