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Human papillomavirus (HPV) related Oropharynx Cancer in the United Kingdom – An evolution in the understanding of disease aetiology

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Declaration of Interests

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Abstract

Substantial increases in oropharyngeal squamous cell carcinoma (OPSCC) incidence have been observed across the developed world and attributed to the rising influence of the Human papillomavirus (HPV) on disease aetiology. This pan-UK multicentre, cross-sectional study aimed to accurately determine the proportion of HPV-positive and -negative OPSCC within the United Kingdom population. Archival tumour-tissue blocks from 1602 patients, previously diagnosed with OPSCC (2002-2011), were collated from eleven centres. HPV status was determined using three validated commercial tests, providing valid data for 1474 cases. Corresponding national incidence data from the same decade were obtained from UK Cancer registries.

The overall proportion of OPSCC caused by HPV between 2002-2011 was 51.8% (95% CI:49.3, 54.4) and this remained unchanged throughout the decade (unadjusted risk ratio:1.00 (95% CI:0.99, 1.02). However, over the same period, the incidence of OPSCC in the broader UK population underwent a 2-fold increase (age standardised rate (ASR) 2002:2.1 (95% CI:1.9, 2.2); 2011:4.1(95% CI:4.0, 4.3)).

Although the absolute numbers of OPSCC diagnosed within the UK between 2002 and 2011 almost doubled, the proportion of HPV-attributable cases remained static, at approximately 50%. These results provide the first evidence that, in the UK, the rapidly increasing incidence of OPSCC cannot be solely attributable to the influence of HPV.

The reasons for this previously undetected parallel increase in HPV-positive and -negative disease warrants further investigation so that appropriate future prevention strategies for both HPV-positive and -negative disease can be implemented.

Introduction

The developed world has experienced a dramatic rise in oropharyngeal squamous cell carcinoma (OPSCC) incidence.(1,2) In England, 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) between 1995 and 2011.(3) Associations between tobacco and alcohol consumption and OPSCC are well established,(4) however sexual behaviour is also a risk factor, with lifetime number of oral sex partners recognised as the behavioural measure most strongly associated with OPSCC development.(5) Changes in sexual behaviour appear to underlie the increasing proportion of OPSCC attributable to oncogenic Human papillomavirus (HPV).(1,4,6) Several North American and European studies 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.(7-11)

In the UK, the proportion of OPSCC attributable to HPV has been assessed in several single-centre studies, however each was small, applied diverse methodology and had restricted geographical coverage.(12-14) The current pan-UK study aimed to assess the proportion of OPSCC attributable to HPV infection in a large contemporary sample (2002-2011 inclusive) using robust, standardised methods. There is a pressing need for these data to facilitate health economic analyses and to inform evidence-based policy-making with regard to prophylactic male HPV vaccination, as has recently been implemented in Australia.(15,16)

Methods

Case selection

The study received Research Ethics Committee approval (REC 11/NQ/0452). Northern Ireland samples were accessed under approvals from the Northern Ireland Biobank (NIB 11/001). OPSCC was

defined as cancers involving the 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 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 used for each block. Tissue microarrays (TMA) were constructed for p16 immunohistochemistry (IHC) and high risk HPV DNA in-situ hybridisation (ISH) testing as previously described.(17) Following construction, haematoxylin and eosin-stained sections of the TMAs were analysed to confirm accuracy of sampling. Samples were considered adequate only if all three TMA cores included tumour.

For PCR, DNA was extracted from 2x10 μm FFPE whole sections by digestion for 16 hours in Tris50mM/EDTA1mM/Tween0.5% with 1mg/mL Proteinase K at 56°C, followed by heat inactivation (100°C for 5 mins) and centrifugation. Extracted DNA was tested for HPV DNA presence 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 positive control and water negative control).

p16 IHC was undertaken as a surrogate marker of HPV oncogene expression(18) 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 malignant cells.(18) 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. This test detects high-risk HPV genotypes 16,18,31,33,35,39,45,51,52,56,58 and 66 and tumours were scored as positive if any blue reaction product co-localised with the nuclei of malignant cells.(19) Focal specific staining of only part of the tumour section was regarded as positive. Diffuse staining of tumour and stromal tissues, representative of non-specific chromogen precipitate, was scored as negative. Pale staining limited to the nucleoli of cells and staining of occasional leucocytes and stromal cells was 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. For quality assurance, the panel undertook a calibration exercise on a training set of OPSCC cases (whole sections and TMAs) prior to scoring the study material.

<u>HPV status classification</u>

All cases were tested using the three HPV-detection 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.[19,20] The results of individual HPV diagnostic tests, and clinically relevant combinations of tests are also reported.

<u>Incidence Data</u>

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); laryngeal (C32); and mouth cancers (IARC definition, comprising C03, gum; C04, floor of mouth; C05, palate [excluding C05.1, soft palate and C05.2, uvula]; and C06, other and unspecified parts of mouth), as representative head and neck subsites not associated with HPV infection.[20] ASR were calculated using the updated 2013 European Standard Population, and mid-2012 UK population estimates.[21]

<u>Statistical analysis</u>

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. Characteristics were compared with excluded cases, and with 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.(22) This approach was used because 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 sample characteristics. Finally, HPV-positive proportions determined in the current study were applied to the UK 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, College Station, TX, USA).

Results

Case characteristics

Figure 1 illustrates the application of study inclusion criteria and the HPV diagnostic testing algorithm. Valid results were obtained for 1474 cases obtained from 11 centres. 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 tonsil cancers. 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 S1). The study sample included 8.3% of the 17739 OPSCC diagnosed in the UK 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 S2).

HPV Prevalence

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%). HPV prevalence also varied between 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.(12-14)

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 remaining 22 samples contained DNA of more than one HPV type. Among cases in which 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 S3). 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.

Incidence of cancers of the oropharynx, larynx and mouth 2002-11, and estimated HPV-associated OPSCC disease burden

Between 2002 and 2011 the UK incidence of OPSCC increased by 100.6%, while the incidence of laryngeal cancer increased by 9.3% (Figure 3). The majority of OPSCC occurred in men (2011 ASR: 6.3 male vs 2.1 female). Laryngeal cancers were similarly more common in men than women (2011 ASR: 7.1 vs 1.3). The incidence of mouth cancers increased by 45.4%, and there was a smaller difference in incidence between men and women than was observed with OPSCC and laryngeal cancers (2011 ASR: 3.7 vs 2.4).

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 increasing incidence of both HPV-positive and negative OPSCC, especially in men. It was notable that incidence curves for HPV-negative OPSCC and mouth cancers show very similar trends. With regard to non-HPV associated head and neck cancers in males, substantial absolute increases in ASR were observed for HPV-negative OPSCC, and for mouth cancers, with a smaller increase in laryngeal cancers (1.72, 1.14, and 0.73 /100,000 respectively).

Discussion

This first nation-wide study investigating the prevalence of HPV in OPSCC within the UK showed that 51.8% (95% CI:49.3, 54.4) of cases diagnosed between 2002 and 2011 were HPV-positive. Over the same period, the incidence of OPSCC in the broader population approximately doubled (ASR 2002:2.1, ASR 2011:4.1), whilst 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 and show that, in the UK at least, the increasing incidence of OPSCC, cannot be explained solely by an increase in HPV-associated disease.

The strengths of the study include: large sample size; broad geographical representation; rigorous and systematic case selection; and, use of well-validated commercial tests to identify HPV tumour status. The results are likely to reflect national trends, although there is potential for variation in OPSCC incidence, and in HPV prevalence, between different geographical areas in the UK. To assess potential bias in case selection, the records supplied by each centre were formally reviewed. This showed that FFPE blocks from only nine patients were unavailable due to use in other clinical studies. The study group included a higher proportion of younger patients (45-54.9 yrs) relative to the OPSCC population, but 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 and 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.

It is important to stress that conclusions based on the data presented should not be generalized beyond the UK. Substantial variation has been reported in the proportion of OPSCC attributable to HPV between countries and time periods.(10,11) This is likely to be a reflection of variations in

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multiple factors, including sexual behaviour and rates of genital HPV infection, as well as tobacco and alcohol consumption. This highlights that trends in the aetiology of OPSCC must be considered in a population-specific manner. 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%).(12-14) 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 previous data, detailing an increased proportion of HPV-positive OPSCC associated with increasing incidence of OPSCC overall.(8) Recent data from North America(10) and Stockholm,(23) however, suggest that plateaus in the proportion of HPV-positive OPSCC and HPV-positive tonsillar squamous cell carcinoma 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, implies that HPV-negative OPSCC, traditionally associated primarily with smoking(24), is also increasing in incidence. However, this contrasts with the more modest increase in incidence of other smoking-related head and neck malignancies, such as laryngeal cancer,(25) (Figure 3) 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,(26,27) but while the current UK tonsillectomy rate is approximately 75% lower than in the 1950s,(28) the absence of a disproportionate increase in OPSCCs specifically involving the tonsils, in our results and other published data,(25) suggests this is not a major contributory factor to the increasing incidence of OPSCC. The observed increases in ASR among the different subsites (non-HPV OPSCC, mouth, and laryngeal) may reflect the degree of exposure of specific anatomical sites to individual carcinogens, including alcohol and tobacco smoke. In an analysis of Dutch HNSCC incidence, van Monsjou *et al.*(29) suggested that behavioural changes in the post-World War II generation included reduced smoking rates coupled with significant rises in alcohol consumption and

they suggested that excessive alcohol intake may be a more critical risk factor for OPSCC than smoking. In the UK, smoking rates have declined from 46% of adults in 1974 to 19% in 2013(30). However, since 1950, per capita alcohol consumption has increased from 3.9 L/year to a peak of 9.4 L/year in 2004(31). This appears consistent with increased incidence of cancers at sites with greater exposure to alcohol e.g mouth, but smaller increases at sites more strongly associated with tobacco smoking e.g. larynx.

It is probable that gender-neutral prophylactic HPV vaccination could prevent HPV-positive OPSCC.(32) Indeed national bodies, such as the Joint Committee on Vaccination and Immunisation in the UK, are currently considering extending prophylactic HPV vaccination to include boys, as well as girls. Our data substantially expand the evidence base available to inform decisions such as this, particularly when viewed in the context of a projected substantial continued rise in OPSCC incidence of up to 239% in the next 20 years.(33) The current bivalent and quadrivalent HPV vaccines protect against infection with 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 in our study. Our data suggest that of the 1781 cases of OPSCC diagnosed in men in the UK in 2011, approximately 926 were HPV-positive, and 866 were associated with HPV types included in current vaccines.

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. The data presented highlight that in the UK, increases in OPSCC incidence are not entirely due to HPV-associated disease. However, these findings should not be extrapolated to other developed world populations, rather, they emphasise the need to assess the aetiology of head and neck cancers, and oropharyngeal cancers in particular, on a population specific basis.

Acknowledgements

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Tables

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	

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

Legends for Tables & Figures:

Table 1: Characteristics of included/excluded cases

[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

[a]HPV prevalence defined according to tier-wise algorithm.
[b]NOS – Not Otherwise Specified.

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, larynx and mouth (UK, 2002-2011)
OPSCC indicates Oropharyngeal Squamous Cell Carcinoma; LSCC indicates Laryngeal Squamous Cell Carcinoma; Mouth comprises malignant neoplasms of the gum, floor of mouth, palate (excluding soft palate and uvula), and other and unspecified parts of the mouth.

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. Incidence of cancers of the mouth is also shown.

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                          ┌─────────────────────┐
                          │   Cases requested   │
                          │      n=1710         │
                          └──────────┬──────────┘
                                     ↓
                          ┌─────────────────────┐       ┌──────────────────────────┐
                          │   Cases supplied    │       │ Samples excluded n = 73  │
                          │      n=1602         │──────→ │  - Non-Oropharynx        │
                          └──────────┬──────────┘       │    Subsite (n= 65)       │
                                     │                  │  - Outside study         │
                                     ↓                  │    inclusion dates       │
                          ┌─────────────────────┐       │    (n= 8)                │
                          │ Cases for HPV testing│       └──────────────────────────┘
                          │      n = 1529       │
                          └──────────┬──────────┘
```

Figure 1 study schema flowchart.

| p16 IHC positive n = 805 | p16 IHC negative n = 683 | p16 IHC invalid n = 41 |

| HPV ISH positive n = 618 | HPV ISH negative n = 178 | HPV ISH invalid n = 9 |

| HPV PCR positive n = 146 | HPV PCR negative n = 27 | HPV PCR invalid n = 5 |

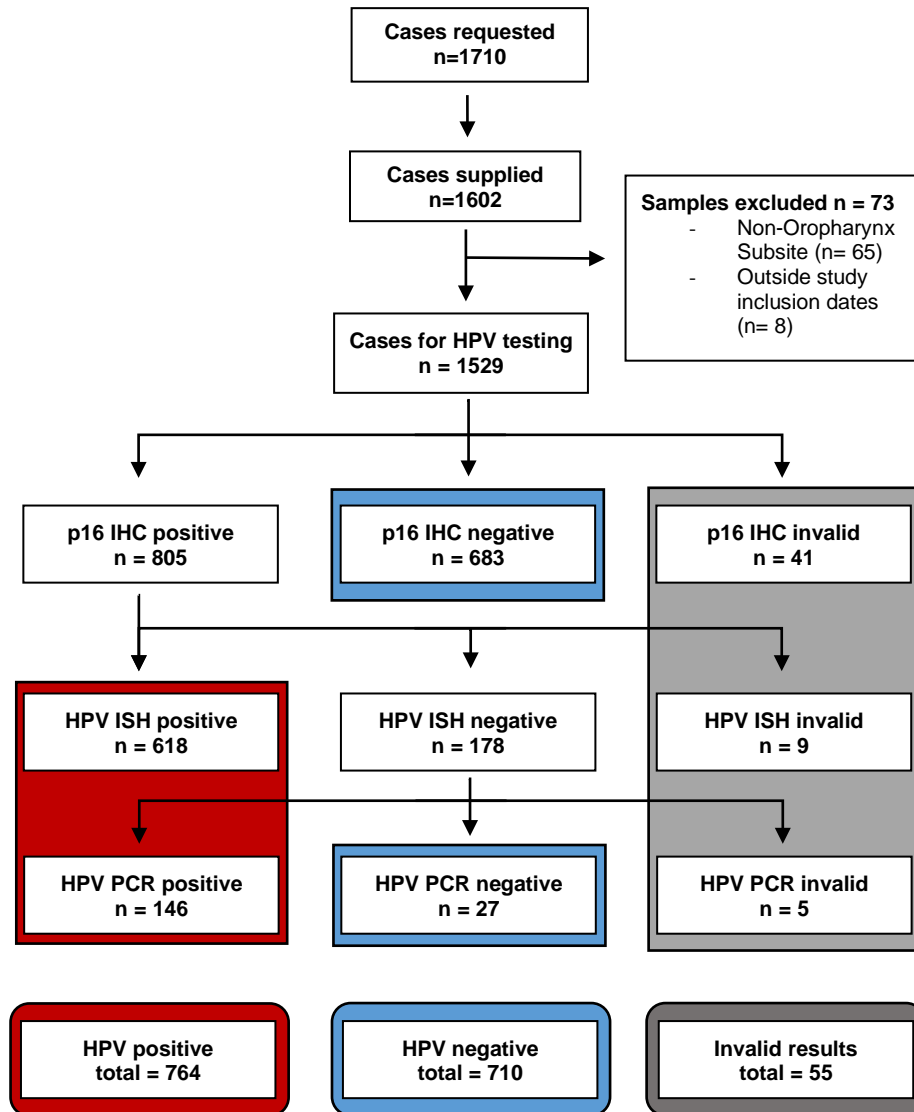| HPV positive total = 764 | HPV negative total = 710 | Invalid results total = 55 |

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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).
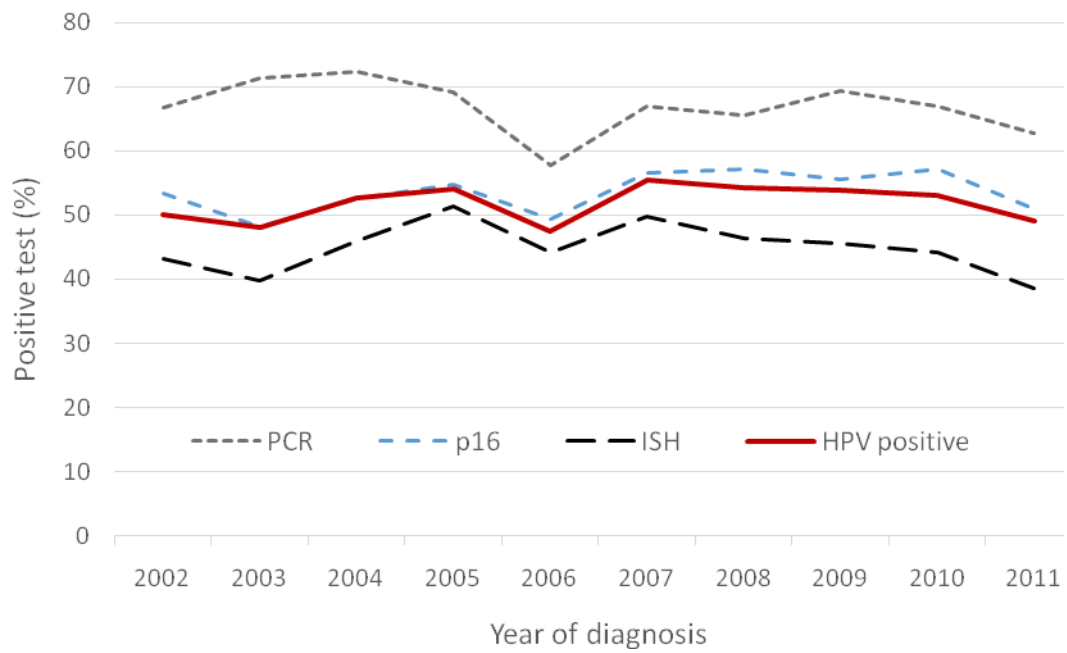
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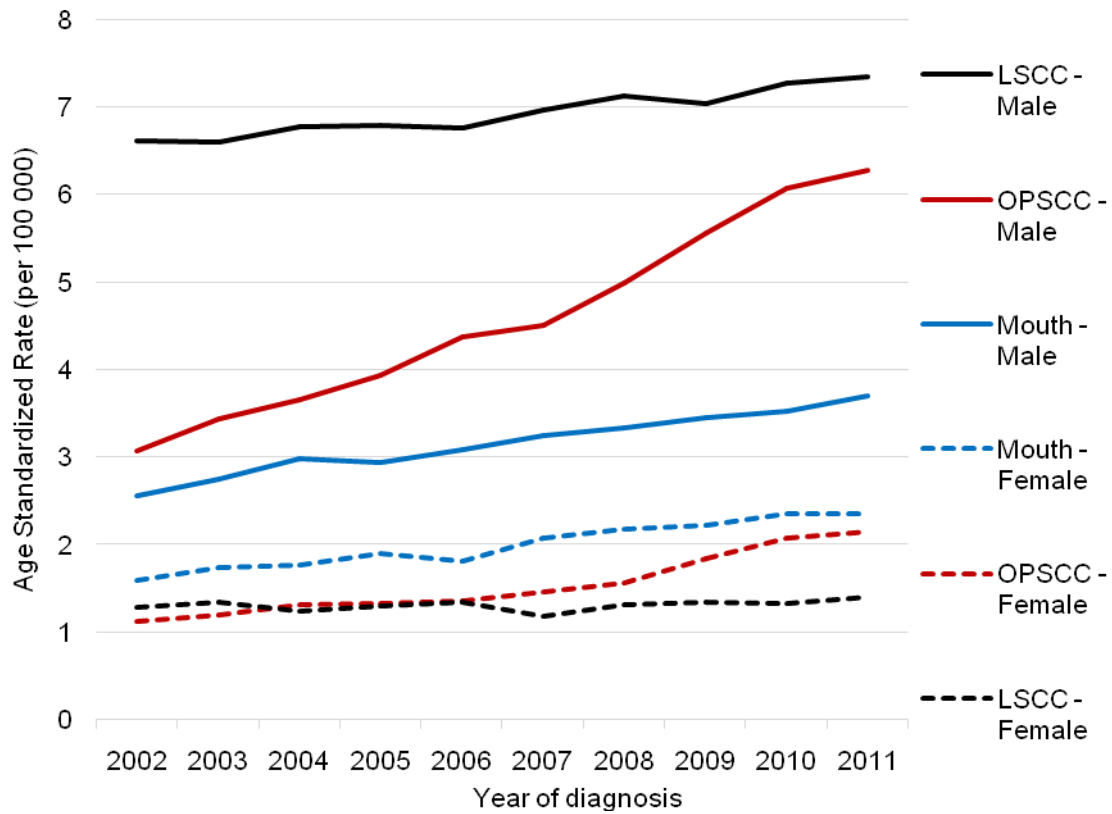
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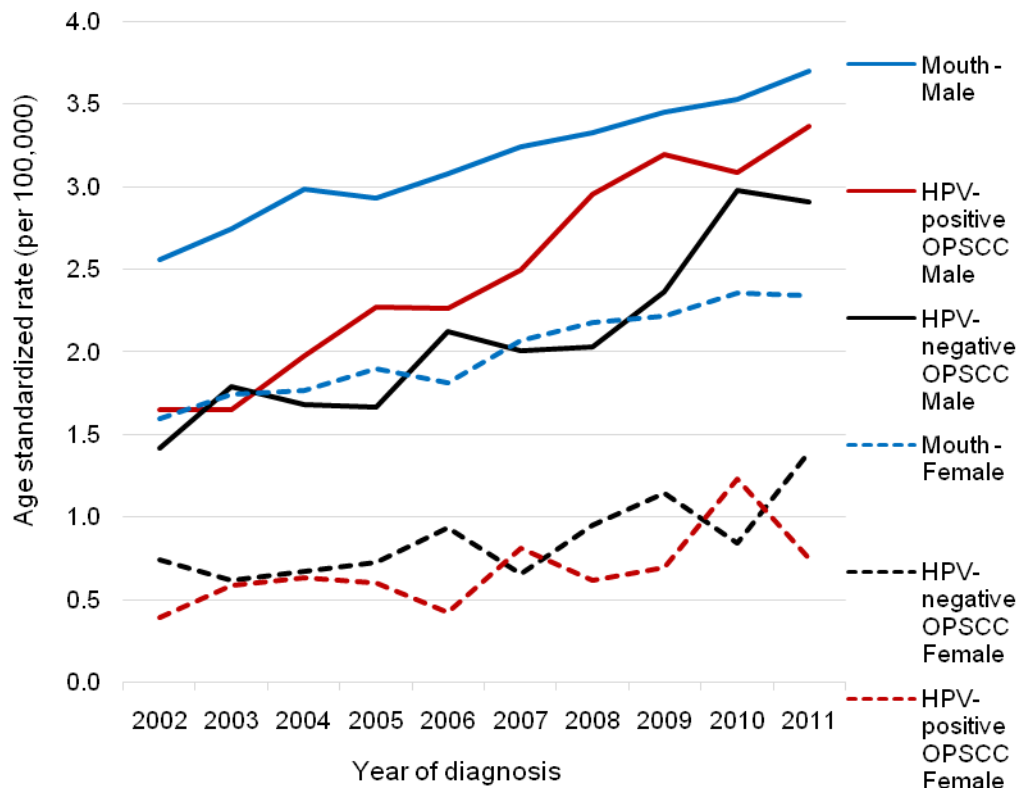
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For each year, the gender-specific proportion of HPV positive samples was multiplied by the gender-specific incidence to estimate ASR for both HPV positive and negative OPSCC. Incidence of cancers of the mouth is also shown.