# Therapeutic advances in B-cell malignancies

## Free CME-accredited Webinar
## October 3, 2023
## 1.30pm BRT | 5:30pm BST | 6:30pm CET

**Join us for this CME-accredited webinar, where the speakers will use a case-based approach to discuss the treatment optimization of B-cell malignancies based on the latest guideline recommendations.**

**There will be the opportunity to ask questions directly to the speaker during the live Q&A or you can send your questions prior to the event. Register today to submit your questions!**

**The talks will focus on:**

- **How to deal with challenging scenarios around toxicities and drug resistance with novel agents**
- **When to consider treatment intensification or combinations**

**REGISTER NOW**

This educational virtual event has been supported by Eli Lilly.

WILEY

Received: 26 April 2023 | Revised: 17 July 2023 | Accepted: 31 July 2023

DOI: 10.1002/ijc.34710

RESEARCH ARTICLE

Tumor Markers and Signatures

IJC INTERNATIONAL JOURNAL of CANCER

# Diagnostic accuracy of HPV16 early antigen serology for HPV-driven oropharyngeal cancer is independent of age and sex

Johannes M. A. Kusters[1,2,3] | Brenda Diergaarde[4] | Andrew Ness[5,6] | Maarten F. Schim van der Loeff[2,7] | Janneke C. M. Heijne[1,8] | Lea Schroeder[3] | Katrina Hueniken[9] | James D. McKay[10] | Gary J. Macfarlane[11] | Pagona Lagiou[12] | Areti Lagiou[13] | Jerry Polesel[14] | Antonio Agudo[15,16] | Laia Alemany[16,17] | Wolfgang Ahrens[18] | Claire M. Healy[19] | David I. Conway[20] | Max Robinson[21] | Christina Canova[22] | Ivana Holcátová[23] | Lorenzo Richiardi[24] | Ariana Znaor[10] | Miranda Pring[6] | Steve Thomas[6] | D. Neil Hayes[25] | Geoffrey Liu[9,26] | Rayjean J. Hung[26,27] | Paul Brennan[10] | Andrew F. Olshan[28] | Shama Virani[10] | Tim Waterboer[3]

**Correspondence**
Tim Waterboer, Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.
Email: t.waterboer@dkfz-heidelberg.de

**Funding information**
Associazione Italiana per la Ricerca sul Cancro, Grant/Award Number: CPDA057222; Canadian Cancer Society Research Institute; Cancer Research UK Programme Grant, the Integrative Cancer Epidemiology Programme, Grant/Award Number: C18281/A19169; Division of Cancer Prevention, National Cancer Institute, Grant/Award Number: R01-CA90731; European Commission, Grant/Award Number: QLK1-2001-00182; National Institute for Health Research, Grant/Award Number: RP-PG-0707-10034; National Institute of Dental and Craniofacial Research, Grant/Award Number: R01 DE025712; National Institutes of Health,

## Abstract

A growing proportion of head and neck cancer (HNC), especially oropharyngeal cancer (OPC), is caused by human papillomavirus (HPV). There are several markers for HPV-driven HNC, one being HPV early antigen serology. We aimed to investigate the diagnostic accuracy of HPV serology and its performance across patient characteristics. Data from the VOYAGER consortium was used, which comprises five studies on HNC from North America and Europe. Diagnostic accuracy, that is, sensitivity, specificity, Cohen's kappa and correctly classified proportions of HPV16 E6 serology, was assessed for OPC and other HNC using $p16^{INK4a}$ immunohistochemistry (p16), HPV in situ hybridization (ISH) and HPV PCR as reference methods. Stratified analyses were performed for variables including age, sex, smoking and alcohol use, to test the robustness of diagnostic accuracy. A risk-factor analysis based on serology was conducted, comparing HPV-driven to non-HPV-driven OPC. Overall, HPV serology had a sensitivity of 86.8% (95% CI 85.1-88.3) and specificity of 91.2% (95% CI

**Abbreviations:** AF, attributable fraction; AIC, Akaike Information Criterion; AJCC, American Joint Committee on Cancer; BMI, body mass index; CI, confidence interval; DNA, deoxyribonucleic acid; HNC, head and neck cancer; HPV, human papillomavirus; ICD-10, International Classification of Diseases Volume; ISH, in situ hybridization; MFI, median fluorescence intensity; OPC, oropharyngeal cancer; OR, odds ratio; p16, $p16^{INK4a}$ immunohistochemistry; PCR, polymerase chain reaction; RNA, ribonucleic acid.

**Disclaimer:** Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organisation, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organisation.

For affiliations refer to page 12

Grant/Award Numbers: P30CA047904, P50CA097190; Princess Margaret Hospital Foundation; University Hospitals Bristol NHS Foundation Trust

88.6-93.4) for HPV-driven OPC using p16 as a reference method. In stratified analyses, diagnostic accuracy remained consistent across sex and different age groups. Sensitivity was lower for heavy smokers (77.7%), OPC without lymph node involvement (74.4%) and the ARCAGE study (66.7%), while specificity decreased for cases with <10 pack-years (72.1%). The risk-factor model included study, year of diagnosis, age, sex, BMI, alcohol use, pack-years, TNM-T and TNM-N stage. HPV serology is a robust biomarker for HPV-driven OPC, and its diagnostic accuracy is independent of age and sex. Future research is suggested on the influence of smoking on HPV antibody levels.

**KEYWORDS**
diagnostic accuracy, head and neck cancer, human papillomavirus, oropharyngeal cancer, serology

**What's new?**

Human papillomavirus (HPV) is driving an increasing proportion of head and neck cancers (HNC), and patients with HPV-driven cancers may have different treatment needs. Serum antibodies against HPV16 early antigens appear to be a highly sensitive biomarker for HPV-driven HNC. Here, the authors investigate the accuracy of HPV early antigen serology across populations of varying characteristics, including age and sex. Overall, they found that HPV serology showed high sensitivity and specificity which was retained independent of age, sex, and other characteristics.

## 1 | INTRODUCTION

With over 900 000 new annual cases and 450 000 deaths worldwide,[1] head and neck cancer (HNC) is a serious health concern. HNC comprises a diverse group of cancers, including those of the oral cavity, pharynx, larynx and sinonasal cavity.[2,3] Traditionally, the main causes of HNC have been long-term tobacco use and alcohol consumption. More recently, it has been recognised that oncogenic human papillomavirus (HPV) types, especially HPV16, cause a subset of HNC as well.[2,4] Much remains unclear about the progression of HPV infection to HNC. It is suggested that patients with HPV-driven HNC are slightly younger than those with non-HPV-driven HNC.[4] HPV-driven HNC incidence varies between countries and populations, as it is more common in North America and Northern Europe, more common among men, and has an increasing incidence in recent decades.[4,5]

HPV-driven HNC has a unique biology, pathology and clinical features.[6,7] Therefore, patients with HPV-driven HNC might have different treatment needs[8]: HPV-positive HNC patients usually respond better to radiotherapy and chemotherapy.[6,9] For oropharyngeal cancers (OPC) the difference in prognosis is even stronger, for which an increase in HPV-driven cancer is seen in recent years.[10] The five-year survival of HPV-positive OPC is 70% to 80% compared to 25% to 40% for HPV-negative OPC.[6,7] Whether the difference in survival is due to molecular pathogenesis, or related to age and overall health of patients is unclear.[9] Additionally, HPV-positive cancer patients are less likely to experience recurrence of OPC.[6] Therefore, being able to distinguish HPV-driven HNC from non-HPV-driven HNC is of clinical and epidemiological importance.

Several methods are available to define whether an HNC is HPV-driven, each with their own strengths and limitations. HPV DNA detection by polymerase chain reaction (PCR) only suggests the presence of viral DNA, but does not identify biological activity of the virus driving carcinogenic processes.[11-13] The detection of both HPV DNA and RNA by PCR is considered more reliable, although these methods are laborious and most clinical routine laboratories might not have access to both DNA and RNA PCR.[11,12] HPV DNA detection by in situ hybridisation (ISH) not only detects the presence of HPV, but also the location of the viral genome in the host cell.[14] Nevertheless, ISH tends to have a lower sensitivity, than for example PCR, and is prone to cross-contamination.[12,15] A widely used marker is $p16^{INK4a}$ immunohistochemistry (p16). The HPV E7 protein degrades the retinoblastoma tumour suppressor protein, leading to the overexpression of p16.[13] Therefore, p16 is used as a surrogate marker for active HPV. This inexpensive method is easy to interpret, well studied, became the primary method in routine clinical diagnostics for OPC,[13,14] and has further been incorporated into the most recent version of the HNC staging.[16] However, this surrogate marker is not HPV-specific, resulting in suboptimal specificity, especially outside the oropharynx.[12-14,17]

Serum antibodies against HPV16 early antigens have emerged as a highly sensitive and specific blood-based biomarker to distinguish HPV-driven from non-HPV-driven HNC, especially for OPC.[11] The main advantages of this method include that it only requires a blood draw, not tumour tissue, that it is less susceptible to cross-contamination, and it can be used in epidemiological studies where tumour tissues are not available.[11] Serology could additionally be used

as a rapid screening method for subsites of the oropharynx that are not routinely tested, particularly when attributable fractions (AFs) are low. A recent systematic review showed that HPV16 E6 serology had the best overall performance, with a sensitivity of 83.1% and specificity of 92.5%,[11] making this the best single antibody marker for HPV-driven OPC. In some rare cases, HPV-driven OPC cases are HPV16 E6 negative, but positive for other HPV16 early (E) antibodies, such as E1, E2 or E7.[18] Based on this, OPC can be also considered HPV-driven when having E6 seropositivity (>1000 MFI), or seropositivity to at least three E proteins.[18] It is unclear whether the sensitivity and specificity of this blood-based biomarker varies by patient characteristics, such as age and sex. Immune responses differ in ageing men and women, which might result in a different diagnostic accuracy.[19,20] Therefore, our study, based on data from an international consortium, aimed to determine the diagnostic accuracy of HPV16 early antigen serology as marker for HPV-driven HNC, and to estimate robustness of the diagnostic accuracy across population characteristics such as age and sex.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

For our study, data from the NIH-funded VOYAGER (Human Papillomavirus, Oral and Oropharyngeal Cancer Genomic Research) consortium were used. This consortium consists of five studies on HNC patients across 10 countries in North America and Europe: (a) the Alcohol-Related Cancers and Genetic Susceptibility in Europe (ARCAGE) study, (b) the Carolina Head and Neck Cancer Study (CHANCE), (c) Head and Neck 5000 (HN5000), (d) the University of Pittsburgh head and neck cancer case-control study (Pittsburgh), and (e) the Mount Sinai Hospital-Princess Margaret (MSH-PMH) study in Toronto (Toronto). The details of these studies have been described previously.[21-25] Four are case-control studies[21-23,25] and one is a case-series.[24] For our analyses, we only used data from HNC cases. In all studies, demographic and lifestyle information were obtained via administered questionnaires. In this analysis, only HNC cases with serology data available were included.

### 2.2 | Tumour classifications

All cancers were classified based on the International Classification of Diseases Volume 10 (ICD-10). Cancers were classified as HNC if they were at the oropharynx (including base of tongue/lingual tonsil), larynx, oral cavity and lip (including salivary glands), nasopharynx, hypopharynx and sinonasal cavities. Other sites (eg, oesophagus, thyroid or jaw) were excluded from our analysis. If the tumour had overlapping sites within the head and neck region without a clear designation of the primary site, it was labelled as having overlapping sites. Finally, tumours were labelled as unspecified if the location was within the head and neck region, but the exact location was unclear (eg, tongue, not otherwise specified).

Tumour stage was classified using the seventh edition of the cancer staging system manual of the American Joint Committee on Cancer (AJCC).[26,27] This staging is based on the tumour size (TNM-T), regional lymph node involvement (TNM-N) and metastasis (TNM-M). We discussed using the new AJCC eighth edition and decided that this would not be appropriate for these analyses given that the new edition already incorporated HPV status into its staging classification.[28]

### 2.3 | HPV serology procedures

All serological analyses were conducted at the German Cancer Research Center (DKFZ, Heidelberg, Germany). HPV antibodies were measured using multiplex serology, a bead-based assay that allows analysis of large numbers of serum samples for antibodies against multiple viral antigens simultaneously.[29] Antibodies against the major capsid protein (L1), early oncoproteins (E6, E7), and regulatory early proteins (E1, E2) were measured for six oncogenic HPV-types: HPV16, HPV18, HPV31, HPV33, HPV45 and HPV52. Antibody levels were further dichotomised into positive and negative, based on predefined cut-offs for median fluorescence intensity (MFI) units (Table S3).[18,29,30]

### 2.4 | Markers of HPV-driven tumours

In our analysis, we used three reference methods to determine if HNC was HPV-driven: (a) p16 immunohistochemistry on tumour tissue was used in patient subsets of all five studies; local determinations for p16 positivity followed standard guidelines; (b) ISH was used in subsets of both the HN5000 study and the Pittsburgh study populations; and (c) PCR was used in a subset of the HN5000 study population. The selection of methods per patient was done as part of the patient's care, so not specifically for our study. More details can be found in Figure 1 and Tables S1 and S2.

Finally, HPV serology was determined for all participants. Tumours were considered to be HPV-driven based on serology (ie, HPV seropositive) if HPV16 E6 was positive (with cut-off >1000 MFI), or at least three HPV16 E-proteins were positive (using a lower cut-off >484 MFI for E6); serological results not meeting the aforementioned data but meeting quality control were considered HPV seronegative.[18]

### 2.5 | Statistical analysis

Study population characteristics were described in relation to HPV serology using descriptive statistics, for the combined study population and for each individual study. HPV AFs were calculated per anatomical location, according to HPV serology.

The overall diagnostic accuracy, as measured by sensitivity, specificity, Cohen's kappa and correctly classified proportion with corresponding 95% confidence intervals (CIs), were calculated for OPC

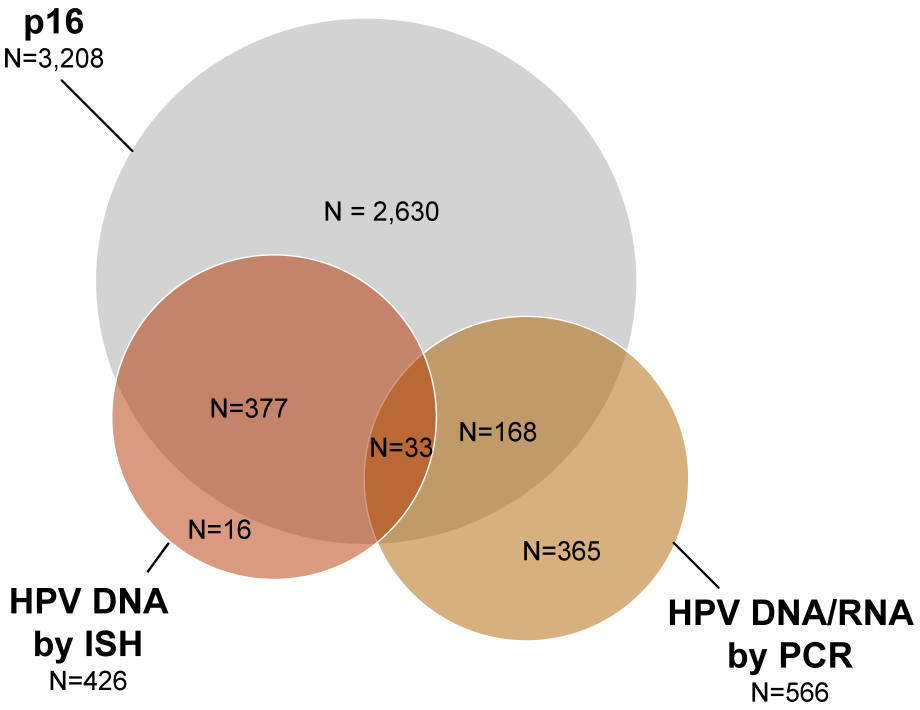


**FIGURE 1** Venn diagram of the study population having had $p16^{INK4a}$ immunohistochemistry, HPV DNA by ISH and/or HPV DNA and RNA by PCR. DNA, deoxyribonucleic acid; HPV, human papillomavirus; ISH, in situ hybridisation; p16, $p16^{INK4a}$ immunohistochemistry; PCR, polymerase chain reaction; RNA, ribonucleic acid.

with p16, ISH and PCR as reference methods, separately. Additionally, a combination of markers was used as a reference, as this is sometimes used in clinical settings; if both p16 and either ISH or PCR were positive, an OPC was considered to be HPV-driven. Cohen's kappa was considered low if the kappa value was below 0.20, fair between 0.20 and 0.40, moderate between 0.40 and 0.60, high between 0.60 and 0.80, and very high if kappa values were higher than 0.80.[31] To evaluate appropriateness of HPV serology to assess HPV-attributability of tumours at other anatomic locations than the oropharynx, diagnostic accuracy was also calculated for cancer at the larynx and oral cavity, using only p16 status as reference test, as this was the only test with sufficient participants. Finally, this was also done for sub-entities of the oropharynx, being the tonsils and base of the tongue.

To assess variation in diagnostic accuracy across patient characteristics, stratified analyses were conducted. Estimates were stratified for variables potentially related to HPV-driven OPC (compared to non-HPV-driven OPC based on HPV serology), being: year of diagnosis (categories based on tertiles), age in categories of 5 years, sex, body mass index (BMI) status at diagnosis, current alcohol use, pack-years, TNM-T, TNM-N and TNM-M stage. Additionally, estimates were stratified by study. For the stratified analyses, p16 was used as a reference method.

Finally, among OPC patients, univariable and multivariable logistic regression models were used to identify risk factors for HPV-driven (based on HPV serology) OPC, compared to non-HPV-driven OPC. For the multivariable model, backwards selection was done based on the Akaike Information Criterion (AIC). Independent variables considered for the model are the same variables as for the stratified diagnostic accuracy, and were tested on collinearity before entering the model with help of a multicollinearity matrix. No strong collinearity was observed. All statistical analyses were conducted using RStudio (Version 1.3.959). For all analyses, statistical significance was defined as $P < .05$.

## 3 | RESULTS

### 3.1 | Study population

The study population comprised 6809 participants with HNC diagnosed between 2002 and 2017, of which 2234 (32.8%) were HPV seropositive (Table 1). The majority of the participants were male (76.6%), ever smoker (65.3%) and consumed alcohol (57.6%). The median age of the study participants was 60.5 years old (interquartile range: 53.1-68.0). The HN5000 study contributed the most participants (60.5%). Characteristics per study are presented in Table S1.

OPC was the most common HNC in our dataset, with 3266 cases (48.0%), followed by oral cavity (24.0%) and laryngeal cancer (18.4%). Of the HPV seropositive participants, 94.6% had OPC (Table 1). Table 2 presents an overview of the anatomical locations, with HPV AFs based on HPV serology. The AF of HPV for OPC was 64.7%. For laryngeal cancer and oral cavity cancer, the HPV AFs were only 1.9% and 3.3%, respectively (Table 2).

### 3.2 | Overall diagnostic accuracy of HPV serology for OPC

Table 3 presents the overall diagnostic accuracy of HPV serology to detect whether an OPC is HPV-driven or not, using three reference methods. Across all three reference methods, HPV serology had a high sensitivity. Against p16 as a reference, sensitivity was 86.8%,

**TABLE 1** Characteristics of the VOYAGER study population, consisting of patients with HNC diagnosed between 2002 and 2017, according to HPV serology.

| Variables | Total, n (%) | Sero−, n (%) | Sero+, n (%) | *P*-value |
|---|---|---|---|---|
| Total | 6809 (100.0) | 4575 (67.2) | 2234 (32.8) | |
| Study | | | | <.001 |
| ARCAGE | 945 (13.9) | 874 (19.1) | 71 (3.2) | |
| CHANCE | 509 (7.5) | 333 (7.3) | 176 (7.9) | |
| HN5000 | 4119 (60.5) | 2934 (64.1) | 1185 (53.0) | |
| Pittsburgh | 371 (5.4) | 129 (2.8) | 242 (10.8) | |
| Toronto | 865 (12.7) | 305 (6.7) | 560 (25.1) | |
| Geographical location | | | | <.001 |
| Continental Europe[a] | 848 (12.5) | 792 (17.3) | 56 (2.5) | |
| North America[b] | 1745 (25.6) | 767 (16.8) | 978 (43.8) | |
| United Kingdom | 4216 (61.9) | 3016 (65.9) | 1200 (53.7) | |
| Year of diagnosis[c] | | | | <.001 |
| 2002-2011 | 2182 (32.0) | 1557 (34.0) | 625 (28.0) | |
| 2012-2013 | 2664 (39.1) | 1802 (39.4) | 862 (38.6) | |
| 2014-2017 | 1963 (28.8) | 1216 (26.6) | 747 (33.4) | |
| Age in years[d] | 60.5 (53.1, 68.0) | 62.0 (54.7, 70.0) | 57.9 (52.0, 64.0) | <.001 |
| Sex | | | | <.001 |
| Male | 5215 (76.6) | 3372 (73.7) | 1843 (82.5) | |
| Female | 1594 (23.4) | 1203 (26.3) | 391 (17.5) | |
| BMI at diagnosis in kg/m[e] | 25.9 (23.1, 29.4) | 25.1 (22.3, 28.6) | 27.4 (24.5, 31.0) | <.001 |
| Unknown/NA | 1326 | 1008 | 318 | |
| Current alcohol use | | | | <.001 |
| Yes | 3923 (57.6) | 2657 (58.1) | 1266 (56.7) | |
| No | 1791 (26.3) | 1100 (24.0) | 691 (30.9) | |
| Unknown/NA | 1095 (16.1) | 818 (17.9) | 277 (12.4) | |
| Drink intensity, units per day[d] | 2.6 (1.2, 5.7) | 3.4 (1.3, 5.9) | 2.0 (0.9, 3.5) | <.001 |
| Unknown/NA | 2515 | 1679 | 836 | |
| Smoking status | | | | <.001 |
| Current smoker | 1793 (26.3) | 1498 (32.7) | 295 (13.2) | |
| Former smoker | 2651 (38.9) | 1666 (36.4) | 985 (44.1) | |
| Never smoker | 1227 (18.0) | 575 (12.6) | 652 (29.2) | |
| Unknown/NA | 1138 (16.7) | 836 (18.3) | 302 (13.5) | |
| Smoking packyears[d] | 22.5 (0.0, 43.0) | 30.0 (8.0, 48.0) | 7.0 (0.0, 29.5) | <.001 |
| Unknown/NA | 1837 | 1363 | 474 | |
| Anatomical location tumour | | | | <.001 |
| Hypopharynx | 262 (3.8) | 245 (5.4) | 17 (0.8) | |
| Larynx | 1254 (18.4) | 1230 (26.9) | 24 (1.1) | |
| Nasopharynx | 102 (1.5) | 89 (1.9) | 13 (0.6) | |
| Oral cavity | 1635 (24.0) | 1581 (34.6) | 54 (2.4) | |
| Oropharynx | 3266 (48.0) | 1152 (25.2) | 2114 (94.6) | |
| Sinonasal | 97 (1.4) | 94 (2.1) | 3 (0.1) | |
| Overlapping sites | 27 (0.4) | 26 (0.6) | 1 (0.0) | |
| Unspecified | 166 (2.4) | 158 (3.5) | 8 (0.4) | |
| TNM-T | | | | <.001 |
| Tx/Tis[f] | 198 (2.9) | 169 (3.7) | 29 (1.3) | |
| T0 | 23 (0.3) | 12 (0.3) | 11 (0.5) | |

(Continues)

**TABLE 1** (Continued)

| Variables | Total, n (%) | Sero−, n (%) | Sero+, n (%) | *P*-value |
|---|---|---|---|---|
| T1 | 1828 (26.8) | 1322 (28.9) | 506 (22.6) | |
| T2 | 2304 (33.8) | 1380 (30.2) | 924 (41.4) | |
| T3 | 1110 (16.3) | 703 (15.4) | 407 (18.2) | |
| T4 | 1346 (19.8) | 989 (21.6) | 357 (16.0) | |
| TNM-N | | | | <.001 |
| Nx[f] | 209 (3.1) | 188 (4.1) | 21 (0.9) | |
| N0 | 2991 (43.9) | 2722 (59.5) | 269 (12.0) | |
| N1 | 763 (11.2) | 481 (10.5) | 282 (12.6) | |
| N2 | 2702 (39.7) | 1125 (24.6) | 1577 (70.6) | |
| N3 | 144 (2.1) | 59 (1.3) | 85 (3.8) | |
| TNM-M | | | | <.001 |
| Mx[f] | 321 (4.7) | 293 (6.4) | 28 (1.3) | |
| M0 | 6409 (94.1) | 4224 (92.3) | 2185 (97.8) | |
| M1 | 79 (1.2) | 58 (1.3) | 21 (0.9) | |
| P16 status | | | | <.001 |
| Negative | 1201 (37.4) | 1149 (75.6) | 52 (3.1) | |
| Positive | 2007 (62.6) | 370 (24.4) | 1637 (96.9) | |
| Unknown/NA | 3601 | 3056 | 545 | |
| HPV DNA by ISH status | | | | <.001 |
| Negative | 129 (30.3) | 84 (74.3) | 45 (14.4) | |
| Positive | 297 (69.7) | 29 (25.7) | 268 (85.6) | |
| Unknown/NA | 6383 | 4462 | 1921 | |
| HPV DNA/RNA by PCR status | | | | <.001 |
| Negative | 299 (52.8) | 287 (80.4) | 12 (5.7) | |
| Positive | 267 (47.2) | 70 (19.6) | 197 (94.3) | |
| Unknown/NA | 6243 | 4218 | 2025 | |

*Note*: *P*-value based on $\chi^2$-test or Wilcoxon rank sum test; Sero−: HPV serology negative; Sero+: HPV serology positive; Unspecified or overlapping sites: HNC cancer without a clear primary; BMI not overweight: <25; BMI overweight: ≥25 and <30; BMI obese: ≥30; TNM classification is a classification to describe tumour size, lymph node involvement and metastasis.

Abbreviations: BMI, body mass index; DNA, deoxyribonucleic acid; HNC, head and neck cancer; HPV, human papillomavirus; ISH, in situ hybridisation; NA, not available; PCR, polymerase chain reaction; RNA, ribonucleic acid.

[a]Countries for Continental Europe: Croatia, Czech Republic, Germany, Greece, Ireland, Italy and Spain.

[b]Countries for North America: Canada, United States of America.

[c]Categories based on tertiles.

[d]*Median* (IQR).

[f]Tx/Nx/Mx: diagnosed with HNC, but T, N or M status not assessed.

**TABLE 2** HPV attributable fractions in HNC cases, according to HPV serology.

| Anatomical location tumour | Totals n (%) | Sero- n (%) | Sero+ n (%) |
|---|---|---|---|
| Oropharynx | 3266 (100) | 1152 (35.3) | 2114 (64.7) |
| Oral cavity | 1635 (100) | 1581 (96.7) | 54 (3.3) |
| Larynx | 1254 (100) | 1230 (98.1) | 24 (1.9) |
| Hypopharynx | 262 (100) | 245 (93.5) | 17 (6.5) |
| Nasopharynx | 102 (100) | 89 (87.3) | 13 (12.7) |
| Sinonasal | 97 (100) | 94 (96.9) | 3 (3.1) |
| Unspecified or overlapping sites | 193 (100) | 184 (95.3) | 9 (4.7) |

*Note*: Unspecified or overlapping sites: HNC cancer without a clear primary. Sero−: HPV serology negative; Sero+: HPV serology positive.

**TABLE 3** Diagnostic accuracy of HPV serology for HPV-driven OPC, compared to the reference methods p16 status, HPV DNA by ISH and HPV DNA/RNA by PCR.

| *Oropharyngeal cancer* | | | | | | |
|---|---|---|---|---|---|---|
| | **p16−** | **p16+** | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **Cohen's kappa (95% CI)** | **Correctly classified proportion (95% CI)** |
| Sero− | 510 | 241 | 86.8 (85.1-88.3) | 91.2 (88.6-93.4) | 0.70 (0.67-0.73) | 87.8 (86.4-89.1) |
| Sero+ | 49 | 1580 | | | | |
| | **ISH−** | **ISH+** | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **Cohen's kappa (95% CI)** | **Correctly classified proportion (95% CI)** |
| Sero− | 58 | 27 | 90.6 (86.6-93.7) | 58.0 (47.7-67.8) | 0.51 (0.41-0.61) | 82.2 (78.0-85.9) |
| Sero+ | 42 | 260 | | | | |
| | **PCR−** | **PCR+** | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **Cohen's kappa (95% CI)** | **Correctly classified proportion (95% CI)** |
| Sero− | 45 | 35 | 83.8 (78.2-88.4) | 93.8 (82.8-98.7) | 0.62 (0.51-0.72) | 85.6 (80.8-89.6) |
| Sero+ | 3 | 181 | | | | |
| | **Combined−** | **Combined+** | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **Cohen's kappa (95% CI)** | **Correctly classified proportion (95% CI)** |
| Sero− | 75 | 39 | 90.2 (86.8-92.9) | 67.0 (57.4-75.6) | 0.48 (0.57-0.65) | 85.1 (81.7-88.1) |
| Sero+ | 37 | 358 | | | | |

*Note*: Sero−: HPV serology negative; Sero+: HPV serology positive. Combined: If for OPC patients both P16 and either ISH or PCR was positive, then positive, otherwise negative.

Abbreviations: DNA, deoxyribonucleic acid; HPV, human papillomavirus; ISH, in situ hybridisation; OPC, oropharyngeal cancer; PCR, polymerase chain reaction; RNA, ribonucleic acid.

against ISH 90.6% and against PCR 83.8%. Specificity of HPV serology was also high against p16 (91.2%) and PCR (93.8%), but lower against ISH (58.0%). With the combined markers as a reference, HPV serology had a sensitivity of 90.2% and specificity of 67.0%.

Only a subset of patients received either an ISH test or p16 test, and the availability of each test was not random. Thus, OPC cases with ISH results might have differed from cases with p16 results. Therefore, a sensitivity analysis was conducted for OPC patients who had both p16 and ISH test results (n = 375), which resulted in similar diagnostic accuracy as the overall estimate (Table S5). Unfortunately, due to a small sample size, the corresponding comparison was not possible to interpret for individuals with both p16 and PCR test results.

Diagnostic accuracy for serology in tonsillar cancer and cancer of base of the tongue was similar to the overall estimates (Table S5). Finally, Table S5 also shows the diagnostic accuracy of HPV serology for larynx cancer and oral cavity cancer with p16 as a reference method, showing a low sensitivity for both anatomical locations.

## 3.3 | Stratified diagnostic accuracy analyses

The stratified diagnostic accuracy was determined for participants with OPC, for which both HPV serology and p16 were available (details of this subpopulation in Table S4). Of the 3266 OPC cases, there were 2380 (72.9%) for whom p16 stats was available. Information on ISH and PCR was available for a much smaller number of study participants, 387 and 264, respectively and could therefore not be used in the stratified analyses.

Stratified diagnostic accuracy analyses were performed for strata of several variables (see Figure 2 and Table S6). Sensitivity estimates did not vary substantially across the age and sex strata. Additionally, they were consistent across all strata of year of diagnosis, BMI status at diagnosis, current alcohol use and TNM-T stage, with sensitivities ranging from 82.6% to 89.6% for those strata, which is similar to the overall estimate against p16 (86.8%). Sensitivities across the studies were inconsistent, with the ARCAGE study, involving cases from Europe, showing a lower sensitivity of 66.7%. Additionally, a lack of confirmed lymph node involvement (TNM—Nx-N0) resulted in a drop of sensitivity to 74.4%, compared to the 90.0% sensitivity in participants with documented lymph node involvement (TNM—N1-N3). Finally, among smokers with 40 or more packyears, sensitivity was lowered to 77.7%.

For specificity, similar estimates were seen across the strata for study, year of diagnosis, age, sex, BMI status at diagnosis, current alcohol use, TNM-T and TNM-N. Specificities ranged from 85.1% to 98.6% for those strata, which was similar to the overall estimate of 91.2% (Figure 2, Table S6). The specificity differed between the smoking strata, with a lower specificity of 72.1% in the stratum of <10 packyears.

Cohen's kappa varied from 0.42 (for individuals with <10 packyears of smoking) to 0.80 (for participants who had TNM—N1-N3 disease). Of the 30 strata examined, 27 had a kappa of at least 0.60. Correctly classified proportions were all considered high, ranging from 80.8% to 92.1% (Table S5).

## 3.4 | Risk-factor model

Univariable and multivariable logistic regression models were generated for all participants with OPC with HPV serology (n = 3266;


**FIGURE 2** Forest plot of the overall, and stratified analyses, for sensitivity and specificity of HPV serology to detect HPV-driven OPC. For these analyses, $p16^{INK4a}$ immunohistochemistry was used as reference method. Categories for year of diagnosis are based on tertiles. BMI not overweight: <25; BMI overweight: ≥25 and <30; BMI obese: ≥30. $T_x/N_x$, diagnosed with OPC, but T or N status not assessed. BMI, body mass index; CI, confidence interval; OPC, oropharyngeal cancer.

Table 4). The univariable logistic regression model showed increasing odds ratios (OR) for having an HPV-driven OPC when having a more recent year of diagnosis, being of lower age, being a male, having a higher BMI at diagnosis, and having a higher TNM-N score. A decreased OR was seen for OPC patients who had greater pack-years and higher TNM-T and TNM-M stages.

**TABLE 4** Risk factors for HPV-driven OPC vs non-HPV-driven OPC according to HPV serology, results from univariable and multivariable analyses.

| | | Unadjusted OR | | Adjusted OR | |
|---|---|---|---|---|---|
| **Variable** | **n/N** | **OR** | **(95% CI)** | **OR** | **(95% CI)** |
| Study | | | | | |
| ARCAGE | 66/237 | 0.18 | (0.13-0.24) | 0.27 | (0.17-0.41) |
| CHANCE | 171/304 | 0.59 | (0.46-0.76) | 0.58 | (0.39-0.86) |
| HN5000 | 1097/1599 | 1 | 1 | 1 | 1 |
| Pittsburgh | 238/320 | 1.33 | (1.02-1.75) | 1.00 | (0.7-1.45) |
| Toronto | 542/806 | 0.94 | (0.78-1.13) | 0.77 | (0.6-0.98) |
| Year of diagnosis[a] | | | | | |
| 2002-2011 | 599/1098 | 1 | 1 | 1 | 1 |
| 2012-2013 | 813/1172 | 1.89 | (1.59-2.24) | 1.39 | (1.05-1.85) |
| 2014-2017 | 702/996 | 1.99 | (1.66-2.38) | 1.48 | (1.11-1.96) |
| Age in years | | | | | |
| <50 | 353/517 | 1.91 | (1.46-2.50) | 1.80 | (1.32-2.45) |
| 50-54 | 421/577 | 2.39 | (1.84-3.13) | 2.22 | (1.64-3.02) |
| 55-59 | 474/707 | 1.81 | (1.41-2.31) | 1.84 | (1.38-2.44) |
| 60-64 | 389/602 | 1.62 | (1.26-2.09) | 1.61 | (1.21-2.15) |
| 65-69 | 255/444 | 1.20 | (0.92-1.57) | 1.17 | (0.86-1.59) |
| ≥70 | 222/419 | 1 | 1 | 1 | 1 |
| Sex | | | | | |
| Male | 1744/2610 | 1.56 | (1.31-1.85) | 1.52 | (1.23-1.86) |
| Female | 370/656 | 1 | 1 | | |
| BMI at diagnosis[b] | | | | | |
| Not overweight | 522/1047 | 1 | 1 | 1 | 1 |
| Overweight | 733/1013 | 2.63 | (2.19-3.17) | 2.12 | (1.72-2.61) |
| Obese | 569/728 | 3.60 | (2.91-4.47) | 2.72 | (2.14-3.47) |
| Unknown/NA | 290/478 | 1.55 | (1.25-1.93) | 1.56 | (0.99-2.46) |
| Current alcohol use | | | | | |
| Yes | 1199/1849 | 1 | 1 | 1 | 1 |
| No | 663/1003 | 1.06 | (0.90-1.24) | 0.98 | (0.8-1.19) |
| Unknown/NA | 252/414 | 0.84 | (0.68-1.05) | 0.53 | (0.29-0.98) |
| Packyears | | | | | |
| <10 pack-years | 701/819 | 1 | 1 | 1 | 1 |
| 10-39 pack-years | 642/1044 | 0.27 | (0.21-0.34) | 0.35 | (0.27-0.45) |
| ≥40 pack-years | 503/978 | 0.18 | (0.14-0.22) | 0.21 | (0.16-0.27) |
| Unknown/NA | 268/425 | 0.29 | (0.22-0.38) | 0.47 | (0.25-0.89) |
| TNM-T | | | | | |
| Tx/Tis[c] | 27/57 | 0.42 | (0.24-0.73) | 2.10 | (0.56-9.99) |
| T0 | 10/15 | 0.94 | (0.33-3.05) | 0.60 | (0.18-2.18) |
| T1 | 480/706 | 1 | 1 | 1 | 1 |
| T2 | 894/1272 | 1.11 | (0.91-1.36) | 1.10 | (0.87-1.39) |
| T3 | 378/621 | 0.73 | (0.58-0.92) | 0.80 | (0.61-1.04) |
| T4 | 325/595 | 0.57 | (0.45-0.71) | 0.53 | (0.4-0.69) |
| TNM-N | | | | | |
| Nx[c] | 19/53 | 0.94 | (0.52-1.67) | 1.51 | (0.31-5.89) |
| N0 | 230/618 | 1 | 1 | 1 | 1 |

(Continues)

**TABLE 4** (Continued)

| Variable | n/N | Unadjusted OR | | Adjusted OR | |
|---|---|---|---|---|---|
| | | OR | (95% CI) | OR | (95% CI) |
| N1 | 272/428 | 2.94 | (2.28-3.8) | 3.18 | (2.4-4.23) |
| N2 | 1511/2045 | 4.77 | (3.95-5.78) | 4.34 | (3.5-5.38) |
| N3 | 82/122 | 3.46 | (2.31-5.26) | 2.99 | (1.9-4.78) |
| TNM-M | | | | | |
| Mx[c] | 26/77 | 0.27 | (0.16-0.42) | | |
| M0 | 2068/3147 | 1 | 1 | | |
| M1 | 20/42 | 0.47 | (0.26-0.87) | | |

*Note*: Sero−: HPV serology negative; Sero+: HPV serology positive; BMI not overweight: <25; BMI overweight: ≥25 and <30; BMI obese: ≥30; TNM classification is a classification to describe tumour size, lymph node involvement and metastasis. Totals might vary due to missings.
Abbreviations: 95% CI, 95% confidence interval; BMI, body mass index; DNA, deoxyribonucleic acid; HNC, head and neck cancer; HPV, human papillomavirus; ISH, in situ hybridisation; NA, not available; OPC, oropharyngeal cancer; OR, odds ratio; PCR, polymerase chain reaction; RNA, ribonucleic acid.
[a]Categories based on terciles.
[b]BMI in $kg/m^2$.
[c]Tx/Nx/Mx: diagnosed with OPC, but T, N or M status not assessed.

The final multivariable model based on backward selection included the following factors associated with being HPV positive (compared to being HPV negative): study, year of diagnosis, age, sex, BMI at diagnosis, current alcohol use, packyears, TNM-T and TNM-N stage (Table 4). The HN5000 study had the highest odds of having an HPV-driven OPC, followed by Pittsburgh, Toronto, CHANCE and finally ARCAGE. Additionally, increased odds were found for male sex, more recent year of diagnosis, younger age, having a higher BMI and not being a current smoker. Having lymph node involvement (>TNM-N0) also increased the odds of having HPV-driven OPC. TNM-M stage was not included as this did not improve the performance of the multivariable model.

## 4 | DISCUSSION

In our study, the diagnostic accuracy of HPV16 early antigen serology was determined as a marker for HPV-driven HNC. Additionally, the robustness of the diagnostic accuracy across strata of patient characteristics was assessed. HPV serology was found to have good diagnostic accuracy with an overall sensitivity of 86.2% and specificity of 91.2% against p16 as a reference. Although immune responses differ in ageing men and women,[19,20] diagnostic accuracy was robust across age and sex, but also across year of diagnosis, BMI at diagnosis, current alcohol use and primary tumour size. However, diagnostic accuracy was found to be potentially influenced by smoking status and lymph node involvement.

To the best of our knowledge, this is the first study assessing the diagnostic accuracy of HPV serology in a real-world setting in which variation across patient characteristics is assessed. Knowing the variability across these characteristics, such as age or sex, is of great importance for interpretation of these results in clinical or research settings. OPC itself is a relatively uncommon condition and therefore most studies on diagnostic accuracy of markers for molecular HPV tumour stage had rather small sample sizes.[18,32-35] By combining studies, from multiple centres and 10 countries, we were able to assess diagnostic accuracy of HPV serology for HPV-driven OPC in one of the largest study populations so far.

HPV serology showed to have a good diagnostic accuracy against p16, even within the sub-entities of the oropharynx. The overall sensitivity and specificity in our study were slightly lower than the first study defining the HPV16 early antigen serology algorithm as used in our study (sensitivity: 97%; specificity: 98%).[18] Main differences include that their study used HPV RNA PCR as reference method, had a small study sample (HNC n = 214, OPC n = 120), and a larger proportion never smoked (35%). Another study using an HPV serological algorithm also found slightly higher diagnostic accuracy (sensitivity: 93.1%; specificity 96.0%).[32] Our study also had a small sample size (n = 112), used as reference p16 combined with ISH, and excluded indeterminate cases (n = 49). Finally, a systematic review and meta-analysis evaluated only HPV16 E6 as a marker for HPV-driven OPC, and found an overall sensitivity of 83.1% and specificity of 94.6%, results which are similar to our results.[11]

Through stratified analyses, our results suggest a robust diagnostic accuracy for HPV serology across the majority of patient subsets defined by clinicodemographic variables. Nevertheless, a variation in diagnostic accuracy was observed between smoking strata: the sensitivity was lower for higher packyears, and specificity decreased for lower packyears. How this discordance between p16 expression and HPV serology is related to smoking is yet unclear. A possible explanation could be that the antibody response could be influenced by smoking, leading to discordance between HPV serology status and p16 status. Laboratory research found that current smokers have statistically significantly higher HPV viral loads than nonsmokers.[36] Most likely this would have led to higher antibody responses. Using the same cut-offs for smokers and nonsmokers, this could lead to a

classification bias for HPV serology status for borderline cases, as smokers are more likely to surpass the cut-off than nonsmokers. Nevertheless, other studies showed no strong and consistent associations between smoking behaviour and HPV antibody expression.[37,38] Additionally, p16 is suggested to be more strongly overexpressed for nonsmokers than among smokers.[39] This may result in a classification bias for nonsmokers, as they might be falsely classified as being HPV-positive, or for smokers if they are falsely classified as being HPV-negative based on their p16. Therefore, the interaction of smoking and the expression of p16 and/or HPV16 antibodies is recommended to be further studied. If differences are found, cut-offs might be set differently for smokers and nonsmokers.

The slightly lower sensitivity for participants without lymph node involvement, might be due to cancer cells which have metastasized to the lymph nodes are more likely to be exposed to the immune system, in addition to immune cell infiltrates.[40] Consequently, lack of lymph node involvement might be a surrogate for a less pronounced immune response, leading to a slightly lower sensitivity of HPV serology. The specificity, however, was not influenced by the lack of lymph node involvement.

HPV serology in the ARCAGE study demonstrated a lower sensitivity. ARCAGE included a small proportion of HPV seropositive patients, which explains the broad CIs. The proportion of smokers and participants without lymph node involvement among those with HPV-driven tumour is higher in our study compared to the other four studies, which might explain the lowered sensitivity. The association between HPV serology and smoking should be further explored.

We performed a multivariable logistic regression model regression analysis to identify factors within patients with OPC that were associated with being HPV-driven. As HPV-driven HNC prevalence is higher in North America and Northern Europe,[4,5] could explain our findings of the larger odds ratios for studies from the United Kingdom and North America. The proportion of HPV-driven OPC has been increasing over the years,[2] which explains why a more recent year of diagnosis is related to higher chance for OPC to be HPV-driven. This might also explain partially why younger patients might have a greater chance of being HPV-positive through a cohort effect.[4] Another explanation is that HPV-driven OPC is often found in younger patients than non-HPV-diven OPC.[2,41] Additionally, male sex and being a nonsmoker are known risk factors for HPV-driven OPC compared to non-HPV-driven OPC.[2,4] Finally, lymph node involvement increased the odds, and a larger tumour size was associated with lower odds for HPV-driven OPC. The fact that patients with HPV-driven OPC often have smaller primary tumours, and patients with those tumours frequently present with lymph node metastasis,[9,42] is a possible explanation for this.

Some limitations should be mentioned. First, all participants in our study originated from Europe or North America. As HPV prevalence varies around the world, with the highest numbers in North America and Europe,[5] the generalisability of our results outside these regions may be limited, especially since important risk factors such as packyears vary across the world.[43] Additionally, data on sexual behaviour were not available for all studies. In previous studies, earlier age of sexual debut, higher number of lifetime partners, higher number of oral sexual partners, and same-sex contact have been suggested to be risk factors for HPV-driven HNC.[4,44-46] Another important limitation is that p16 was used as a reference for the diagnostic accuracy in most analyses. Although this is the most common method for determining HPV status in OPC clinically and in research, and is widely accepted, it has a lower specificity than desired.[14,17] This might have resulted in an underestimation of the true diagnostic accuracy. In an atempt to adjust for this, a combined marker was used in one of the analyses. Nevertheless, this might have resulted in a biased subset, as only specific patients will have had more than one diagnosis method. In the sensitivity analyses, p16 was also used as a reference method for HNC outside of the oropharynx, as opposed to previous studies suggesting p16 should not be used outside the oropharynx as a prognostic marker for HPV-driven tumours.[47] PCR might have served as a better reference for all anatomical locations, but unfortunately, very few patients across all studies of the VOYAGER consortium had PCR-based results for HPV. Finally, not all participants in our dataset had p16 (or any other marker method) been performed. Therefore, our results might not be generalisable to all OPC or HNC patients. Nevertheless, HPV serology could serve as a feasible substitute for current HPV detection methods Additionally, the diagnostic accuracy was shown to be robust across most risk factors, which is in favour of the generalisability.

In conclusion, in this large multinational and multicentre study, we demonstrated that HPV serology can be used for assessing whether OPC is HPV-driven, due to the high sensitivity and specificity. This marker is suggested to be robust across age and sex, but also across year of diagnosis, BMI, current alcohol use and tumour size. Further research is recommended on the interaction of smoking and p16 expression, and on the interaction of smoking and HPV antibody expression, to explain the variation in sensitivity and specificity. In addition, definitions for seropositivity might be further optimised in further studies for different groups, to increase the diagnostic accuracy even further. Nevertheless, due to the robustness across all other variables, this marker is recommended to be used in clinical and epidemiological settings, especially when other methods are not available. Serology is minimally invasive, and can be used when tumour tissue is not available. Currently, different treatment options are being discussed for HPV-driven OPC patients, for example, by de-intensifying therapy, HPV serology could be a useful tool to distinguish HPV-driven OPC from non-HPV-driven OPC. Finally, more research on HPV serology for other HNC than OPC, should be explored, so that this tool may be used for those sites as well.

## AUTHOR CONTRIBUTIONS

The work reported in the paper has been performed by the authors, unless clearly specified in the text. Conceptualisation: Johannes M. A. Kusters, Tim Waterboer, Brenda Diergaarde, Shama Virani, Andrew Ness. Data curation: Johannes M. A. Kusters, Shama Virani, Brenda Diergaarde. Resources: Brenda Diergaarde, Andrew Ness, Miranda Pring, Steve Thomas, Andrew F. Olshan, Katrina Hueniken, Paul Brennan, Geoffrey Liu, Rayjean J. Hung, Gary J. Macfarlane, Pagona Lagiou, Areti Lagiou, Lorenzo Richiardi, Ariana Znoar, Wolfgang

Ahrens, Christina Canova, David I. Conway. Methodology: Johannes M. A. Kusters, Tim Waterboer, Brenda Diergaarde, Shama Virani. Formal analysis, visualisation and writing original draft: Johannes M. A. Kusters. Writing – review and editing: all co-authors. Supervision: Tim Waterboer, Maarten F. Schim van der Loeff, Janneke C. M. Heijne, Brenda Diergaarde, Shama Virani. All authors contributed to the interpretation of the results and critical revision of the manuscript.

## AFFILIATIONS

[1]Centre for Infectious Diseases Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[2]Institute for Infection and Immunity (AII), Amsterdam UMC, Amsterdam, The Netherlands
[3]Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[4]School of Public Health, University of Pittsburgh and UPMC Hillman Cancer Centre, Pittsburgh, Pennsylvania, USA
[5]NIHR Bristol Biomedical Research Centre, Weston NHS Foundation Trust, University of Bristol, Bristol, UK
[6]Bristol Dental School, University of Bristol, Bristol, UK
[7]Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, The Netherlands
[8]Department of Social Medicine, Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, The Netherlands
[9]Princess Margaret Cancer Centre, Temerty School of Medicine, University of Toronto, Toronto, Ontario, Canada
[10]Genomic Epidemiology Group, International Agency for Research on Cancer, Lyon, France
[11]Epidemiology Group, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK
[12]School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
[13]School of Public Health, University of West Attica, Athens, Greece
[14]National Cancer Institute (IRCCS), Rome, Italy
[15]Unit of Nutrition and Cancer, Catalan Institute of Oncology – ICO, L'Hospitalet de Llobregat, Spain
[16]Nutrition and Cancer Group, Epidemiology Public Health Cancer Prevention and Palliative Care Program, Bellvitge Biomedical Research Institute – IDIBELL, L'Hospitalet de Llobregat, Spain
[17]Centro de Investigación Biomédica en Red: Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[18]University of Bremen, Bremen, Germany
[19]Trinity College School of Dental Science, Dublin, Ireland
[20]School of Medicine, Dentistry, and Nursing, University of Glasgow, Dublin, UK
[21]Department of Cellular Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, UK
[22]University of Padova, Padova, Italy
[23]First Faculty of Medicine, Institute of Hygiene and Epidemiology, Charles University, Prague, Czech Republic
[24]Reference Center for Epidemiology and Cancer Prevention, Piemonte, Italy
[25]Division of Medical Oncology and Center for Cancer Research, University of Tennessee Health Science Center, Memphis, Tennessee, USA
[26]Dalla Lana School of Public Health, University of Toronto, Toronto, Canada
[27]Prosserman Centre for Population Health Research, Lunenfeld-Tanenbaum Research Institute, Sinai Health, Toronto, Ontario, Canada
[28]University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, North Carolina, USA

## FUNDING INFORMATION

This project was funded in part by NIH/NIDCR R01 DE025712 (Paul Brennan, Brenda Diergaarde and Neil Hayes). The Alcohol-Related Cancers and Genetic Susceptibility Study in Europe (ARCAGE) was funded by the European Commission's fifth framework program (QLK1-2001-00182), the Italian Association for Cancer Research, Compagnia di San Paolo/FIRMS, Region Piemonte and Padova University (CPDA057222). We thank Dr. Wolfgang Ahrens, PhD (Universität Bremen, Germany) for his support in ARCAGE study. The Carolina Head and Neck Cancer Epidemiology (CHANCE) study was supported in part by the National Cancer Institute (R01-CA90731). The Head and Neck 5000 study was a component of independent research funded by the National Institute for Health Research (NIHR) under its Programme Grants for Applied Research scheme (RP-PG-0707-10034). The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. Core funding was also provided through awards from Above and Beyond, University Hospitals Bristol and Weston Research Capability Funding and the NIHR Senior Investigator award to Professor Andy Ness. Human papillomavirus (HPV) serology was supported by a Cancer Research UK Programme Grant, the Integrative Cancer Epidemiology Programme (grant number: C18281/A19169). The University of Pittsburgh head and neck cancer case-control study is supported by US National Institutes of Health grants P50CA097190 and P30CA047904. The MSH-PMH study was supported by the Canadian Cancer Society Research Institute and the Lusi Wong Programs at the Princess Margaret Hospital Foundation.

## CONFLICT OF INTEREST STATEMENT

Tim Waterboer serves on advisory boards for Merck, Sharp & Dohme (MSD). L. Alemany Vilches serves as consultant and a member of steerings committees (payment to the institution), and the department of L. Alemany Vilches received sponsorships for grants and contracts from MSD, Seegene, Hologic, GSK and Roche. The institute of Maarten F. Schim van der Loeff receives funding for an investigator-initiated study from GSK, and Maarten F. Schim van der Loeff served on an advisory board of Novosanis (payment to the institution). The other authors do not have a conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

All participants provided written informed consent, and the research protocols of the studies were reviewed and approved by the local institutional review boards of each participating study.

## ORCID

*Johannes M. A. Kusters* https://orcid.org/0000-0002-2509-1661
*Jerry Polesel* https://orcid.org/0000-0001-9381-1520
*David I. Conway* https://orcid.org/0000-0001-7762-4063
*Ariana Znaor* https://orcid.org/0000-0002-5849-4782
*D. Neil Hayes* https://orcid.org/0000-0001-6203-7771
*Rayjean J. Hung* https://orcid.org/0000-0002-4486-7496
*Paul Brennan* https://orcid.org/0000-0002-0518-8714

## REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
2. Chow LQ. Head and neck cancer. *N Engl J Med*. 2020;382:60-72.
3. Mehanna H, Paleri V, West C, Nutting C. Head and neck cancer—part 1: epidemiology, presentation, and prevention. *BMJ*. 2010;341:c4684.
4. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. *J Clin Oncol*. 2015;33:3235-3242.
5. Anantharaman D, Abedi-Ardekani B, Beachler DC, et al. Geographic heterogeneity in the prevalence of human papillomavirus in head and neck cancer. *Int J Cancer*. 2017;140:1968-1975.
6. O'Rorke M, Ellison M, Murray L, Moran M, James J, Anderson L. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol*. 2012;48:1191-1201.
7. Psyrri A, Gouveris P, Vermorken JB. Human papillomavirus-related head and neck tumors: clinical and research implication. *Curr Opin Oncol*. 2009;21:201-205.
8. Cramer JD, Burtness B, Le QT, Ferris RL. The changing therapeutic landscape of head and neck cancer. *Nat Rev Clin Oncol*. 2019;16:669-683.
9. Alsahafi E, Begg K, Amelio I, et al. Clinical update on head and neck cancer: molecular biology and ongoing challenges. *Cell Death Dis*. 2019;10:1-17.
10. Reuschenbach M, Tinhofer I, Wittekindt C, Wagner S, Klussmann JP. A systematic review of the HPV-attributable fraction of oropharyngeal squamous cell carcinomas in Germany. *Cancer Med*. 2019;8:1908-1918.
11. Hibbert J, Halec G, Baaken D, Waterboer T, Brenner N. Sensitivity and specificity of human papillomavirus (HPV) 16 early antigen serology for HPV-driven oropharyngeal cancer: a systematic literature review and meta-analysis. *Cancers*. 2021;13:3010.
12. Venuti A, Paolini F. HPV detection methods in head and neck cancer. *Head Neck Pathol*. 2012;6:63-74.
13. Lewis JS. p16 immunohistochemistry as a standalone test for risk stratification in oropharyngeal squamous cell carcinoma. *Head Neck Pathol*. 2012;6:75-82.
14. Schlecht NF, Brandwein-Gensler M, Nuovo GJ, et al. A comparison of clinically utilized human papillomavirus detection methods in head and neck cancer. *Mod Pathol*. 2011;24:1295-1305.
15. Stevens TM, Caughron SK, Dunn ST, Knezetic J, Gatalica Z. Detection of high-risk HPV in head and neck squamous cell carcinomas: comparison of chromogenic in situ hybridization and a reverse line blot method. *Appl Immunohistochem Mol Morphol*. 2011;19:574-578.
16. Zanoni DK, Patel SG, Shah JP. Changes in the 8th edition of the American Joint Committee on Cancer (AJCC) staging of head and neck cancer: rationale and implications. *Curr Oncol Rep*. 2019;21:1-7.
17. Prigge ES, Arbyn M, von Knebel DM, Reuschenbach M. Diagnostic accuracy of p16INK4a immunohistochemistry in oropharyngeal squamous cell carcinomas: a systematic review and meta-analysis. *Int J Cancer*. 2017;140:1186-1198.
18. Holzinger D, Wichmann G, Baboci L, et al. Sensitivity and specificity of antibodies against HPV16 E6 and other early proteins for the detection of HPV16-driven oropharyngeal squamous cell carcinoma. *Int J Cancer*. 2017;140:2748-2757.
19. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16:626-638.
20. Giefing-Kröll C, Berger P, Lepperdinger G, Grubeck-Loebenstein B. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell*. 2015;14:309-321.
21. Lagiou P, Georgila C, Minaki P, et al. Alcohol-related cancers and genetic susceptibility in Europe: the ARCAGE project: study samples and data collection. *Eur J Cancer Prev*. 2009;18:76-84.
22. Divaris K, Olshan AF, Smith J, et al. Oral health and risk for head and neck squamous cell carcinoma: the Carolina head and neck cancer study. *Cancer Causes Control*. 2010;21:567-575.
23. Troy JD, Grandis JR, Youk AO, Diergaarde B, Romkes M, Weissfeld JL. Childhood passive smoke exposure is associated with adult head and neck cancer. *Cancer Epidemiol*. 2013;37:417-423.
24. Ness AR, Waylen A, Hurley K, et al. Establishing a large prospective clinical cohort in people with head and neck cancer as a biomedical resource: head and neck 5000. *BMC Cancer*. 2014;14:1-6.
25. Kachuri L, Saarela O, Bojesen SE, et al. Mendelian randomization and mediation analysis of leukocyte telomere length and risk of lung and head and neck cancers. *Int J Epidemiol*. 2019;48:751-766.
26. Lydiatt WM, Patel SG, O'Sullivan B, et al. Head and neck cancers—major changes in the American joint committee on cancer eighth edition cancer staging manual. *CA Cancer J Clin*. 2017;67:122-137.
27. Egner JR. AJCC cancer staging manual. *JAMA*. 2010;304:1726-1727.
28. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA: A Cancer Journal for Clinicians*, 2017;67:93-99. https://doi.org/10.3322/caac.21388.
29. Waterboer T, Sehr P, Michael KM, et al. Multiplex human papillomavirus serology based on in situ–purified glutathione S-transferase fusion proteins. *Clin Chem*. 2005;51:1845-1853.
30. Holzinger D, Flechtenmacher C, Henfling N, et al. Identification of oropharyngeal squamous cell carcinomas with active HPV16 involvement by immunohistochemical analysis of the retinoblastoma protein pathway. *Int J Cancer*. 2013;133:1389-1399.
31. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33:159-174.
32. Lang Kuhs KA, Kreimer AR, Trivedi S, et al. Human papillomavirus 16 E 6 antibodies are sensitive for human papillomavirus-driven oropharyngeal cancer and are associated with recurrence. *Cancer*. 2017;123:4382-4390.
33. Anderson KS, Gerber JE, D'Souza G, et al. Biologic predictors of serologic responses to HPV in oropharyngeal cancer: the HOTSPOT study. *Oral Oncol*. 2015;51:751-758.
34. Dahlstrom KR, Anderson KS, Field MS, et al. Diagnostic accuracy of serum antibodies to human papillomavirus type 16 early antigens in the detection of human papillomavirus-related oropharyngeal cancer. *Cancer*. 2017;123:4886-4894.
35. Broglie MA, Jochum W, Michel A, et al. Evaluation of type-specific antibodies to high risk-human papillomavirus (HPV) proteins in patients with oropharyngeal cancer. *Oral Oncol*. 2017;70:43-50.

36. Xi LF, Koutsky LA, Castle PE, et al. Relationship between cigarette smoking and human papilloma virus types 16 and 18 DNA load. *Cancer Epidemiol Biomarkers Prev*. 2009;18:3490-3496.
37. Brenner N, Mentzer AJ, Hill M, et al. Characterization of human papillomavirus (HPV) 16 E6 seropositive individuals without HPV-associated malignancies after 10 years of follow-up in the UK biobank. *EBioMedicine*. 2020;62:103123.
38. Smith EM, Pawlita M, Rubenstein LM, Haugen TH, Hamsikova E, Turek LP. Risk factors and survival by HPV-16 E6 and E7 antibody status in human papillomavirus positive head and neck cancer. *Int J Cancer*. 2010;127:111-117.
39. Dediol E, Sabol I, Virag M, Grce M, Muller D, Manojlović S. HPV prevalence and p16 INK a overexpression in non-smoking non-drinking oral cavity cancer patients. *Oral Dis*. 2016;22:517-522.
40. Goldenberg D, Begum S, Westra WH, et al. Cystic lymph node metastasis in patients with head and neck cancer: an HPV-associated phenomenon. *Head Neck*. 2008;30:898-903.
41. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol*. 2013;31:4550-4559.
42. Ducatman BS. The role of human papillomavirus in oropharyngeal squamous cell carcinoma. *Arch Pathol Lab Med*. 2018;142:715-718.
43. Ng M, Freeman MK, Fleming TD, et al. Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. *JAMA*. 2014;311:183-192.
44. Smith EM, Ritchie JM, Summersgill KF, et al. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. *Int J Cancer*. 2004;108:766-772.
45. Heck JE, Berthiller J, Vaccarella S, et al. Sexual behaviours and the risk of head and neck cancers: a pooled analysis in the international head and neck cancer epidemiology (INHANCE) consortium. *Int J Epidemiol*. 2010;39:166-181.
46. D'Souza G, Kreimer AR, Viscidi R, et al. Case–control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*. 2007;356:1944-1956.
47. Meshman J, Wang P-C, Chin R, et al. Prognostic significance of p16 in squamous cell carcinoma of the larynx and hypopharynx. *Am J Otolaryngol*. 2017;38:31-37.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Kusters JMA, Diergaarde B, Ness A, et al. Diagnostic accuracy of HPV16 early antigen serology for HPV-driven oropharyngeal cancer is independent of age and sex. *Int J Cancer*. 2023;1-14. doi:10.1002/ijc.34710

# B-cell malignancies - A new knowledge hub on the latest research in therapeutic advances

## EDUCATIONAL CONTENT AVAILABLE ON THE HUB:

- On-demand Webinars - earn CME credit
- Infographics
- Patient Case Studies
- Currated Research Articles

...and much more

**VISIT KNOWLEDGE HUB TODAY**

This educational resource has been supported by Eli Lilly.

WILEY