Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multicentre, multinational, individual patient data analysis

Hisham Mehanna, Miren Taberna*, Christian von Buchwald, Sara Tous, Jill Brooks, Marisa Mena, Francisca Morey, Christian Grønhøj, Jacob Høygaard Rasmussen, Martin Garset-Zamani, Laia Bruni, Nikolaos Batis, Ruud H Brakenhoff, C René Leemans, Robert J Baatenburg de Jong, Jens Peter Klussmann, Nora Wuerdemann, Steffen Wagner, Tina Dalianis, Linda Marklund, Haïtham Mirghani, Andrew Schache, Jaqueline A James, Shao Hui Huang, Brian O'Sullivan, Paul Nankivell, Martina A Broglie, Markus Hoffmann, Elgar Susanne Quabius, Laia Alemany, on behalf of the HNCIG-EPIC group*

Summary

Background p16^{INK4a} (p16) immunohistochemistry is the most widely used biomarker assay for inferring HPV causation in oropharyngeal cancer in clinical and trial settings. However, discordance exists between p16 and HPV DNA or RNA status in some patients with oropharyngeal cancer. We aimed to clearly quantify the extent of discordance, and its prognostic implications.

Methods In this multicentre, multinational individual patient data analysis, we did a literature search in PubMed and Cochrane database for systematic reviews and original studies published in English between Jan 1, 1970, and Sept 30, 2022. We included retrospective series and prospective cohorts of consecutively recruited patients previously analysed in individual studies with minimum cohort size of 100 patients with primary squamous cell carcinoma of the oropharynx. Patient inclusion criteria were diagnosis with a primary squamous cell carcinoma of oropharyngeal cancer; data on p16 immunohistochemistry and on HPV testing; information on age, sex, tobacco, and alcohol use; staging by TNM 7th edition; information on treatments received; and data on clinical outcomes and follow-up (date of last follow-up if alive, date of recurrence or metastasis, and date and cause of death). There were no limits on age or performance status. The primary outcomes were the proportion of patients of the overall cohort who showed the different p16 and HPV result combinations, as well as 5-year overall survival and 5-year disease-free survival. Patients with recurrent or metastatic disease or who were treated palliatively were excluded from overall survival and disease-free survival analyses. Multivariable analysis models were used to calculate adjusted hazard ratios (aHR) for different p16 and HPV testing methods for overall survival, adjusted for prespecified confounding factors.

Findings Our search returned 13 eligible studies that provided individual data for 13 cohorts of patients with oropharyngeal cancer from the UK, Canada, Denmark, Sweden, France, Germany, the Netherlands, Switzerland, and Spain. 7895 patients with oropharyngeal cancer were assessed for eligibility. 241 were excluded before analysis, and 7654 were eligible for p16 and HPV analysis. 5714 (74·7%) of 7654 patients were male and 1940 (25·3%) were female. Ethnicity data were not reported. 3805 patients were p16-positive, 415 (10·9%) of whom were HPV-negative. This proportion differed significantly by geographical region and was highest in the areas with lowest HPV-attributable fractions (r=–0·744, p=0·0035). The proportion of patients with p16+/HPV– oropharyngeal cancer was highest in subsites outside the tonsil and base of tongue (29·7% *vs* 9·0%, p<0·0001). 5-year overall survival was 81·1% (95% CI 79·5–82·7) for p16+/HPV+, 40·4% (38·6–42·4) for p16–/HPV–, 53·2% (46·6–60·8) for p16–/HPV+, and 54·7% (49·2–60·9) for p16+/HPV–. 5-year disease-free survival was 84·3% (95% CI 82·9–85·7) for p16+/HPV+, 60·8% (58·8–62·9) for p16–/HPV–; 71·1% (64·7–78·2) for p16–/HPV+, and 67·9% (62·5–73·7) for p16+/HPV–. Results were similar across all European sub-regions, but there were insufficient numbers of discordant patients from North America to draw conclusions in this cohort.

Interpretation Patients with discordant oropharyngeal cancer (p16–/HPV+ or p16+/HPV–) had a significantly worse prognosis than patients with p16+/HPV+ oropharyngeal cancer, and a significantly better prognosis than patients with p16–/HPV– oropharyngeal cancer. Along with routine p16 immunohistochemistry, HPV testing should be mandated for clinical trials for all patients (or at least following a positive p16 test), and is recommended where HPV status might influence patient care, especially in areas with low HPV-attributable fractions.

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*Contributed equally as first authors

Institute of Head and Neck Studies and Education (InHANSE), Institute of Cancer and Genomics Sciences, University of Birmingham, Birmingham, UK (Prof H Mehanna FRCS, J Brooks PhD, N Batis PhD, P Nankivell PhD); **Department of Medical Oncology** (M Taberna PhD) **and Cancer Epidemiology Research Programme** (M Taberna, S Tous BSc, M Mena PhD, F Morey BSc, L Bruni PhD, L Alemany PhD), **Catalan Institute of Oncology (ICO), Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain; Department of Medicine, University of Barcelona, Barcelona, Spain** (M Taberna); **Epidemiology and Public Health, Centro de Investigación Biomédica en Red: Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain** (S Tous, M Mena, L Bruni, L Alemany); **Department of Otolaryngology, Head and Neck Surgery and Audiology, Copenhagen University Hospital—Rigshospitalet, Copenhagen, Denmark** (Prof C von Buchwald MD, Prof C Grønhøj PhD, J H Rasmussen PhD, M Garset-Zamani MD); **Otolaryngology-head and neck surgery, Cancer Centre Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, Netherlands** (Prof R H Brakenhoff PhD,

Prof C R Leemans PhD); **Department of Otolaryngology, Head and Neck Surgery, Erasmus MC Cancer Centre, Rotterdam, Netherlands** (Prof R J Baatengurg de Jong PhD); **Department of Otorhinolaryngology, Head and Neck Surgery, Medical Faculty, University of Cologne, Cologne, Germany** (Prof J P Klussman PhD, N Wuerdemann MD); **Department of Otorhinolaryngology, Head and Neck Surgery, University of Giessen, Giessen, Germany** (S Wagner Dr. rer. nat.); **Department of Oncology-Pathology** (Prof T Dalianis PhD), **Department of Clinical Science, Intervention and Technology, Department of Oto-Rhinolaryngology, Head and Neck Surgery** (Prof L Marklund PhD), **and Medical Unit Head and Neck, Lung and Skin Cancer** (Prof L Marklund), **Karolinska University Hospital, Stockholm, Sweden; Department of Surgical Sciences, Section of Otolaryngology and Head and Neck Surgery, Uppsala University, Uppsala, Sweden** (Prof L Marklund); **Department of Head and Neck Oncology, Gustave Roussy Cancer Campus, Villejuif, France** (Prof H Mirghani PhD); **Liverpool Head & Neck Centre, Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, UK** (A Schache PhD); **Precision Medicine Centre of Excellence, Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, Northern Ireland, UK** (Prof J A James PhD); **Regional Molecular Diagnostic Service, Belfast Health and Social Care Trust, Belfast, Northern Ireland, UK** (Prof J A James); **Department of Radiation Oncology/Otolaryngology—Head and Neck Surgery, Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada** (Prof S H Huang MD, Prof B O'Sullivan MD); **Department of Otorhinolaryngology—Head and Neck Surgery, University Hospital Zurich, University of Zurich, Zurich, Switzerland** (M A Broglie MD); **Department of Otorhinolaryngology, Head and Neck Surgery, Christian-Albrechts-University Kiel, Kiel, Germany** (Prof M Hoffmann MD, E S Quabius MD)

Research in context

Evidence before this study

The incidence of oropharyngeal cancer has increased rapidly worldwide since the year 2000, mainly because of the increase in human papillomavirus (HPV)-mediated disease. Because of the high cost and implementation difficulties of HPV DNA and RNA testing, overexpression of the protein $p^{16INK4a}$ (hereafter referred to as p16) on immunohistochemistry is widely used as a surrogate for determining HPV mediation. However, a large proportion of p16-positive patients are HPV negative, and a large proportion of p16-negative patients are HPV positive (so-called discordant cases). We searched PubMed and the Cochrane database for systematic reviews and original studies published in English between Jan 1, 1970, and Sept 30, 2022, using the search terms "p16, HPV, head neck cancer or oropharynx, concordance or discordant or association". We included retrospective series and prospective cohorts previously analysed in individual studies that reported on concordance between p16 and HPV status, reported on prognosis, and had minimum cohort sizes of 100 patients with primary squamous cell carcinomas of the oropharynx. Our search reveals substantial controversy over the proportion of patients with p16-positive oropharyngeal cancer who are negative on HPV (DNA and RNA) testing (up to 20%). Results on the prognostic significance of this discordant group are contradictory, ranging from reported survival outcomes similar to that in patients with p16-positive and HPV-positive (p16+/HPV+) oropharyngeal cancer, who have excellent prognoses, to outcomes similar to that in patients with p16-negative and HPV-negative (p16–/HPV–) oropharyngeal cancer, who have much worse prognoses. The controversy has continued because of inadequately powered, single-centre studies, which do not account for regional variations. Ascertaining prognosis for discordant patients is important because of the implications for patient counselling, and for deciding which trials to offer these patients (de-escalating treatment intensity to improve functional outcomes for patients with a good prognosis or increasing treatment intensity to improve survival for patients with a poor prognosis).

Added value of this study

To address this issue, this multinational study has collated, to our knowledge, the largest cohort to date (7654 patients from nine countries) and provides sufficient power to elucidate the prognosis of discordant patients. The study confirms that a substantial number of p16-positive patients are actually HPV-negative when tested for HPV DNA or RNA. Moreover, proportions of p16-positive patients who are actually HPV-negative differ significantly by geographic region, with the highest discordant rates in areas with the lowest HPV-attributable fractions (proportions of patients with oropharyngeal cancer caused by HPV).

We also report that discordant patients had prognoses that were significantly worse than that in patients with p16+/HPV+ oropharyngeal cancer, but significantly better than that in patients with p16–/HPV– oropharyngeal cancer.

Implications of all the available evidence

Along with routine p16 immunohistochemistry, HPV testing is strongly recommended where HPV status determines eligibility for clinical trials, where it affects patient counselling, and where treatment de-escalation or intensification are being considered, especially in areas with low HPV-attributable fractions.

Introduction

Around the year 2000, human papillomavirus (HPV) emerged as a new causal agent for oropharyngeal cancer.[1] Since then, the proportion of oropharyngeal cancer cases that are caused by HPV (the HPV-attributable fraction [HPV-AF]), has increased dramatically in some global regions, especially in North America, Europe, and Australia.[2–6]

HPV-mediated oropharyngeal cancer is a distinct disease entity with different epidemiological, molecular, and clinical features, and is characterised by better treatment responsiveness and survival than HPV-unrelated oropharyngeal cancer.[7] Given the prognostic advantage of HPV-related oropharyngeal cancer, the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) developed separate classifications for HPV-related and HPV-unrelated disease in the new TNM 8th edition staging system (TNM 8) for HPV-related oropharyngeal cancer. Several clinical trials have assessed or are currently assessing treatment de-intensification strategies in this disease setting.[8,9]

HPV mediation of oropharyngeal cancer can be ascertained by testing for the presence of HPV DNA or mRNA in the tumour by use of PCR-based methods or in-situ hybridisation. Overexpression of the protein $p16^{INK4a}$ (hereafter referred to as p16) serves as an excellent surrogate biomarker for HPV causation in oropharyngeal cancer[10,11] because the HPV early protein E7 results in p16 overexpression in HPV-related cancers. In HPV-unrelated oropharyngeal cancer, the *CDKN2A* gene encoding p16 is mutated or lost in almost all cases, and so p16 is usually not expressed in these tumours.

Because of its favourable performance as a surrogate biomarker, and the relative ease of application, p16 immunohistochemistry was chosen as the preferred assay for AJCC TNM 8 staging. For that staging system, and for most of the de-escalation clinical trials done so far,[9,12] HPV-related oropharyngeal cancer is therefore usually defined on the basis of overexpression of p16 alone, without other HPV biomarker confirmation.[13] p16 positivity is defined as at least 70% of the tumour cells showing moderate to strong diffuse nuclear and cytoplasmic p16 immunoreactivity on immunohistochemistry.

However, up to 20% of patients who have p16-positive tumours test negative for HPV DNA or RNA.[14,15] In some studies, outcomes in patients with p16-positive, HPV-negative oropharyngeal cancer resembled the improved outcomes of patients with double positive (p16-positive and HPV-positive) cancer, but results of other studies show a poorer prognosis, similar to that in patients with double-negative (p16-negative and HPV-negative) cancer.[15–24] If the poorer prognosis is confirmed, then the use of p16 alone for the TNM 8 staging system and for inclusion in clinical trials of treatment de-escalation might not be appropriate, since patients with p16-positive and HPV-negative cancer, who respond less well to treatment and are at higher risk of recurrence than patients with p16-positive and HPV-positive cancer, would be misclassified as having HPV-related tumours and could undergo de-escalation of treatment, which could be detrimental to their overall survival.[2]

Few studies have described the characteristics and prognosis of patients with discordant combinations of oropharyngeal cancer (ie, p16–/HPV+ or p16+/HPV–). Moreover, many of these studies were based on data from a single centre, included few patients, or described only a single geographical region, resulting in less robustness.[15–24] In collaboration with the Head and Neck Cancer Inter Group, we aimed to define the proportion, characteristics, and prognosis of patients with oropharyngeal cancer with discordant p16 and HPV testing results and ascertain the additional prognostic benefit of combined p16 and HPV testing over p16 testing alone in the clinical setting.

Methods

Study design

This study was a centralised individual patient data analysis on data from 13 cohorts from the UK, Canada, Denmark, Sweden, France, Germany, the Netherlands, Switzerland, and Spain. We did a literature search in PubMed and Cochrane database for systematic reviews and original studies published in English between Jan 1, 1970, and Sept 30, 2022. We included retrospective series and prospective cohorts of consecutively recruited patients previously analysed in individual studies with minimum cohort sizes of 100 patients with primary squamous cell carcinomas of the oropharynx. Inclusion criteria were diagnosis of a primary squamous cell carcinoma of oropharyngeal cancer; data on p16 immunohistochemistry and on HPV testing (by HPV RNA PCR, HPV DNA PCR, HPV DNA in-situ hybridisation, or HPV RNA in-situ hybridisation); information on age, sex, tobacco, and alcohol use; staging by TNM 7th edition; information on treatments received; and data on clinical outcomes and follow-up (date of last follow-up if alive, date of recurrence or metastasis, and date and cause of death). Data on race and ethnicity were not collected. There were no limits on age or performance status. Patients underwent cross-sectional imaging and histological confirmation by biopsy and were treated with surgery, radiotherapy, chemotherapy, or a combination, or were treated palliatively.

Pathological diagnoses were based on formalin-fixed paraffin embedded tissue, fresh frozen tissue, or fresh tissue. All tumours had to have been assessed for p16 expression by immunohistochemistry and only those with strong nuclear and cytoplasmic staining in at least 70% of the tumour cells were considered positive.[13] Information regarding p16, HPV DNA, and RNA evaluation techniques used by each cohort included in the study are summarised in the appendix (pp 1–6).

We addressed potential selection bias by using strict recruitment criteria, requiring consecutively recruited patients, requiring a minimum size of cohort for inclusion, and separating analysis from recruitment of cohorts. Duplicates were excluded. We addressed other forms of bias through sensitivity analysis by statistical methods.

Database

A standardised Microsoft Excel form was used for anonymisation and data harmonisation. The data fields collected can be seen in the appendix (p 29). Each centre completed their excel data form with all the relevant details, and uploaded it using a digitally secure method to a central repository at the University of Birmingham (Birmingham, UK), where data from all the centres were collated and encrypted to enable secure delivery to Institut Català d'Oncologia, Barcelona, Spain, for analysis. Since this was an analysis of anonymised published data, no ethics approval was deemed necessary by the ethics committee of the lead site (Birmingham).

Outcomes

There were two primary outcomes. The first was the proportion (%) of patients in the overall cohort who showed the different p16 and HPV result combinations. The second primary outcome was overall survival (defined as duration from diagnosis to death from any cause, last follow-up appointment, or end of the follow-up period) and disease-free survival (defined as the duration from diagnosis to first recurrence or metastasis observed, last follow-up appointment, or end of the follow-up period). The follow-up period was calculated from the date of diagnosis to death, recurrence, or metastasis (if observed) and restricted to the first 5 years.

Statistical analysis

Analyses were pre-planned in a statistical analysis plan as part of the protocol (appendix). No sample size calculation was done because all patients from the collaborative cohorts were going to be included. For the included variables in the analyses, the proportion of missing values was less than 5%, except for the alcohol data (30% missing, cohort dependent), so no missing imputation was done. Smoking status was defined as never smoker or ever smoker (including current and former smokers). Descriptive analysis of all collected variables was done,

Correspondence to:
Prof Hisham Mehanna, Institute of Head and Neck Studies and Education (InHANSE), Institute of Cancer and Genomics Sciences, University of Birmingham, Birmingham B15 2TT, UK
h.mehanna@bham.ac.uk

See **Online** for appendix

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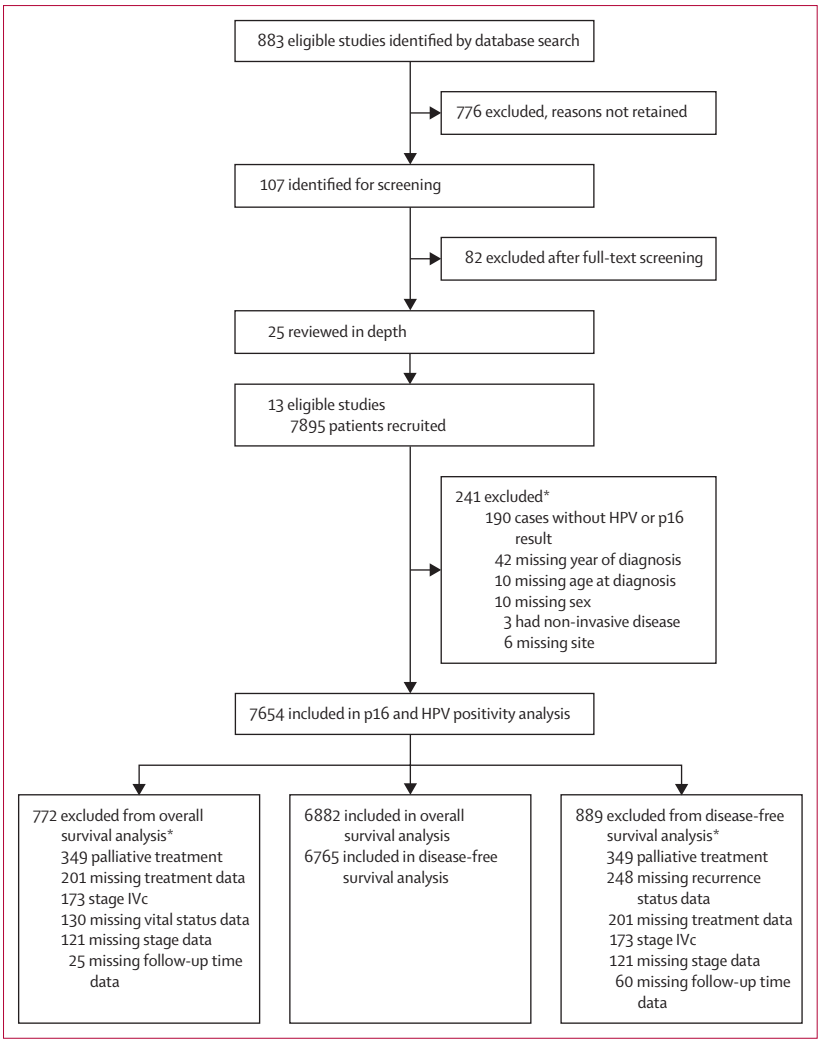


Figure 1: **Trial profile**
HPV=human papillomavirus. *Participants could be excluded for multiple reasons.

and comparisons were done by use of χ^2, Kruskal-Wallis, ANOVA, or log-rank tests, when appropriate. Paired Pearson correlation coefficient (r) was calculated to measure the linear relationship between the proportion of patients with p16+/HPV+ (double-positive) oropharyngeal cancer versus those with p16+/HPV– oropharyngeal cancer. Adjusted multinomial logistic regression analyses were done to identify the determinants for all the p16 and HPV combinations, using p16–/HPV– (double-negative) patients as the reference group.

Adjusted proportional hazards models (Cox regression) were done to estimate the effect of p16 and HPV biomarkers on 5-year overall survival and disease-free survival adjusted by the different potential confounding factors (biomarker combinations, sex, cohort, treatment, stage, year of diagnosis, and age for overall survival and biomarker combinations, stage, sex, year of diagnosis, cohort, and treatment for disease-free survival). Patients with recurrent or metastatic disease or who were treated palliatively were excluded from overall survival and disease-free survival analyses. The planned oropharyngeal cancer-specific survival analysis could not be done because the information needed was not provided for most of the participating cohorts. Proportional hazard assumptions were checked on the basis of both the proportional hazards test of a Cox regression and Schoenfeld residuals and log-likelihood ratio test p-value was used to assess the significance of each variable in the model. All analyses were done as a total, followed by subgroup analyses according to the following effect modifiers: geographical region (North America *vs* northern Europe *vs* western Europe *vs* southern Europe), smoking status (never smokers *vs* ever smokers), and anatomical subsite (tonsil, base of the tongue, tonsil and base of the tongue, or other oropharyngeal sites). Potential bias was addressed by performing sensitivity analyses to examine the effect of HPV testing techniques (DNA PCR, DNA in-situ hybridisation, RNA PCR, and RNA in-situ hybridisation), some centres' testing protocols (appendix pp 10–11), and year of recruitment, by analysing the cohort of patients recruited between 1999 and 2015 to homogenise the recruitment period for all the cohorts (appendix p 16). Analyses were undertaken to exclude the patients provided by France and the Netherlands to account for any bias they could introduce, because they undertook p16 immunohistochemistry first, then did HPV DNA testing only on p16-positive patients, assuming that p16-negative patients would also be HPV-negative (data not shown). To exclude any effects that older cohorts in northern and southern Europe might have on HPV positivity incidence, sensitivity analyses were only done on the cohort of patients recruited between 1999 and 2015 to homogenise the recruitment period for all the cohorts. Adjusted survival curves were drawn for all collected variables to estimate the cumulative probability of survival (overall survival and disease-free survival).

All regression models were adjusted by the following: p16 and HPV status, smoking status, age, sex, period of diagnosis, centre, stage (according to TNM 7th edition), and treatment. Age was the only quantitative variable used and was included as a continuous variable in the survival analysis. It was also included in the multinomial regression model in quintiles to better observe its trend related to the different p16 and HPV combinations. The significance threshold was established initially at 0·05. Bonferroni's correction was used in multiple comparison analyses, ie, reducing the significance level to 0·025 (0·05/2) for two, 0·02 (0·05/3) for three, 0·01 (0·05/4) for four comparisons. The analyses were done using STATA/SE version 16.0, R version 4.2.1, and R Studio (version 1.4.1106).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Our search returned 883 studies, 13 of which met all eligibility criteria and retrieved individual information, including 7895 patients with oropharyngeal cancer recruited between Jan 1, 1988, and Sept 30, 2018. 241 patients were excluded (190 had no HPV or p16 result, three non-invasive cases, six missing site, ten missing age at diagnosis, ten missing sex data, and 42 missing year of diagnosis; participants could be excluded for multiple reasons). 7654 patients were eligible for p16 and HPV positivity analysis (figure 1). 772 patients were excluded from the overall survival analysis (n=6882), and 889 patients were excluded from the disease-free survival analysis (n=6765; appendix p 17).

Median patient age was 60·0 years (IQR 53·0–67·0). 5714 (74·7%) of 7654 patients were male and 1940 (25·3%) were female. 5855 (76·5%) patients were current or former smokers. 1327 (17·3%) patients had early-stage disease at diagnosis (TNM 7 stage I–II), 6033 (78·8%) had locally advanced disease (stage III and IVa–b), and 173 (2·3%) had distant metastatic disease (stage IVc; table 1). Between the 13 cohorts, we observed

	Total (n=7654)	p16–/HPV– (n=3560)	p16–/HPV+ (n=289)	p16+/HPV– (n=415)	p16+/HPV+ (n=3390)
Geographical region (p<0·0001)					
North America	186 (2·4%)	33 (0·9%)	18 (6·2%)	2 (0·5%)	133 (3·9%)
Toronto, ON, Canada	186 (2·4%)	33 (0·9%)	18 (6·2%)	2 (0·5%)	133 (3·9%)
Northern Europe	3964 (51·8%)	1352 (38·0%)	187 (64·7%)	227 (54·7%)	2198 (64·8%)
Copenhagen, Denmark	2169 (28·3%)	755 (21·2%)	90 (31·1%)	123 (29·6%)	1201 (35·4%)
Stockholm, Sweden	539 (7·0%)	125 (3·5%)	39 (13·5%)	24 (5·8%)	351 (10·4%)
Belfast, UK	232 (3·0%)	137 (3·85%)	0	11 (2·7%)	84 (2·5%)
Birmingham, UK	775 (10·1%)	262 (7·4%)	34 (11·8%)	58 (14·0%)	421 (12·4%)
Liverpool, UK	249 (3·3%)	73 (2·1%)	24 (8·3%)	11 (2·7%)	141 (4·2%)
Western Europe	2647 (34·6%)	1445 (40·6%)	52 (18·0%)	158 (38·1%)	992 (29·3%)
Paris, France	275 (3·6%)	0	0	35 (8·4%)	240 (7·1%)
Cologne, Germany	205 (2·7%)	90 (2·5%)	4 (1·4%)	17 (4·1%)	94 (2·8%)
Giessen, Germany	704 (9·2%)	431 (12·1%)	38 (13·1%)	40 (9·4%)	195 (5·8%)
Kiel, Germany	126 (1·6%)	62 (1·7%)	6 (2·1%)	11 (2·7%)	47 (1·4%)
Amsterdam and Rotterdam, Netherlands	1203 (15·7%)	815 (22·9%)	0	48 (11·6%)	340 (10·0%)
Zurich, Switzerland	134 (1·8%)	47 (1·3%)	4 (1·4%)	7 (1·7%)	76 (2·2%)
Southern Europe	857 (11·2%)	730 (20·5%)	32 (11·1%)	28 (6·7%)	67 (2·0%)
Barcelona, Spain	857 (11·2%)	730 (20·5%)	32 (11·1%)	28 (6·7%)	67 (2·0%)
Age at diagnosis, years (p<0·0001)*					
Mean (SD)	60·4 (10·1)	61·3 (9·8)	59·7 (10·9)	60·6 (9·7)	59·5 (10·4)
Median (IQR; range)	60 (53–67; 19–100)	61 (54–68; 19–100)	59 (52–67; 21–91)	60 (54–67; 34–87)	59 (52–66; 27–98)
Sex (p=0·2020)					
Male	5714 (74·7%)	2621 (73·6%)	214 (74·0%)	308 (74·2%)	2571 (75·8%)
Female	1940 (25·3%)	939 (26·4%)	75 (26·0%)	107 (25·8%)	819 (24·2%)
Year of diagnosis					
1988–1994	105 (1·4%)	87 (2·4%)	6 (2·1%)	5 (1·2%)	7 (0·2%)
1995–1999	132 (1·7%)	113 (3·2%)	2 (0·7%)	10 (2·4%)	7 (0·2%)
2000–2004	1636 (21·4%)	961 (27·0%)	57 (19·7%)	85 (20·5%)	533 (15·7%)
2005–2009	2691 (35·2%)	1154 (32·4%)	121 (41·9%)	152 (36·6%)	1264 (37·3%)
2010–2014	2040 (26·7%)	864 (24·3%)	74 (25·6%)	104 (25·1%)	998 (29·4%)
2015–2018	1050 (13·7%)	381 (10·7%)	29 (10·0%)	59 (14·2%)	581 (17·1%)
Smoking status (p<0·0001)					
Never smoker	1470 (19·2%)	200 (5·9%)	48 (17·0%)	73 (18·6%)	1149 (35·3%)
Current or former smoker	5855 (76·5%)	3195 (94·1%)	235 (83·0%)	319 (81·4%)	2106 (64·7%)
Unknown†	329 (4·3%)	165	6	23	135
Alcohol consumption (p<0·0001)					
Never drinker	1352 (17·7%)	460 (16·6%)	45 (23·1%)	68 (27·1%)	779 (36·6%)
Current or former drinker	3993 (52·2%)	2308 (83·4%)	150 (76·9%)	183 (72·9%)	1352 (63·4%)
Unknown†	2309 (30·2%)	792	94	164	1259

(Table 1 continues on next page)

	Total (n=7654)	p16–/HPV– (n=3560)	p16–/HPV+ (n=289)	p16+/HPV– (n=415)	p16+/HPV+ (n=3390)
(Continued from previous page)					
Tumour subsite (p<0·0001)					
Tonsil	4094 (53·5%)	1412 (39·7%)	146 (50·5%)	222 (53·5%)	2314 (68·3%)
Base of the tongue	1765 (23·1%)	794 (22·3%)	55 (19·0%)	88 (21·2%)	828 (24·4%)
Other	1795 (23·4%)	1354 (38·0%)	88 (30·4%)	105 (25·3%)	248 (7·3%)
Stage (TNM 7th edition); (p<0·0001)					
I–II	1327 (17·3%)	848 (24·3%)	47 (16·6%)	61 (15·0%)	371 (11·0%)
III–IVb	6033 (78·8%)	2537 (72·8%)	224 (79·2%)	333 (81·8%)	2939 (87·5%)
IVc	173 (2·3%)	98 (2·8%)	12 (4·2%)	13 (3·2%)	50 (1·5%)
Unknown†	121 (1·6%)	77	6	8	30
Treatment (p<0·0001)					
Surgery	601 (7·9%)	381 (11·1%)	25 (9·2%)	30 (7·5%)	165 (4·9%)
Radiotherapy	1912 (25·0%)	955 (27·8%)	69 (25·5%)	108 (26·9%)	780 (23·4%)
Surgery and radiotherapy or chemotherapy	1704 (22·3%)	712 (20·7%)	61 (22·5%)	89 (22·2%)	842 (25·2%)
Radiotherapy and cetuximab +/– induction	285 (3·7%)	132 (3·8%)	10 (3·7%)	16 (4·0%)	127 (3·8%)
Radiotherapy and cisplatin +/– induction	2602 (34·0%)	1009 (29·3%)	84 (31·0%)	134 (33·4%)	1375 (41·2%)
Palliative	349 (4·6%)	252 (7·3%)	22 (8·1%)	24 (6·0%)	51 (1·5%)
Unknown†	201 (2·6%)	119 (%)	18 (%)	14 (%)	50 (%)
Vital status at 5 years of follow-up (n=6882); (p<0·0001)‡					
Alive	4531 (65·8%)	1424 (46·6%)	144 (60·0%)	226 (62·1%)	2737 (85·0%)
Dead	2351 (34·2%)	1634 (53·4%)	96 (40·0%)	138 (37·9%)	483 (15·0%)
Recurrence/metastasis at 5 years of follow-up (n=6765); (p<0·0001)‡					
No	5262 (77·8%)	2104 (68·9%	173 (76·5%)	270 (74·0%)	2715 (87·0%)
Yes	1503 (22·2%)	950 (31·1%)	53 (23·5%)	95 (26·0%)	405 (13·0%)
HPV detection techniques					
DNA PCR (p<0·0001)	6194 (80·9%)	3110 (87·4%)	252 (87·2%)	304 (74·3%)	2528 (74·6%)
DNA PCR positivity§	2779 (44·9%)	0	252 (100·0%)	0	2527 (100·0%)
RNA PCR (p<0·0001)	214 (2·8%)	39 (1·1%)	30 (10·8%)	5 (1·2%)	140 (4·1%)
RNA PCR positivity§	146 (68·2%)	0	16 (53·3%)	0	130 (92·9%)
DNA in situ hybridisation (p<0·0001)	1610 (21·0%)	472 (13·6%)	58 (20·1%)	114 (27·5%)	966 (28·5%)
DNA in situ hybridisation positivity§	755 (46·9%)	0	7 (12·1%)	0	748 (77·4%)
RNA in situ hybridisation (p<0·0001)	976 (12·8%)	386 (10·8%)	34 (11·8%)	63 (15·2%)	493 (14·5%)
RNA in situ hybridisation positivity§	513 (52·6%)	0	33 (97·1%)	0	480 (97·4%)

Data are n (%) unless otherwise specified. p values obtained for comparisons between each variable with p16/HPV distribution using χ^2 test. HPV=human papillomavirus. *ANOVA test p value. †Missing data category; its contribution not included in the calculation of percentage distribution by p16/HPV groups. ‡Log-rank test p value. §Percentage of HPV positivity among cases tested by each technique.

Table 1: **Clinical and demographic characteristics of patients according to each biomarker combination group**

differences regarding year of diagnosis, tobacco use, and alcohol use (appendix pp 1–6, 8).

Of 7654 patients with samples tested for both p16 and HPV by DNA or mRNA, 3560 (46·5%) were p16–/HPV– (double negative), 3390 (44·3%) were p16+/HPV+ (double positive), 289 (3·8%) were p16–/HPV+, and 415 (5·4%) were p16+/HPV– (table 1). Of the 3805 p16-positive patients, 415 (10·9%) did not show evidence for presence of HPV. Of the 3849 p16-negative patients, 289 (7·5%) showed presence of HPV.

HPV-AF showed a statistically significant increasing trend since 1991 (p<0·0001). This trend was observed in all geographical regions (data not shown). Figure 2 shows the distribution of the p16+/HPV– patient subgroups according to HPV-AFs for each geographical region. Regions with higher HPV-AFs had a lower proportion of patients with p16+/HPV– oropharyngeal cancer (r=–0·744, p=0·0035). North America (Toronto, Canada) was the region with the highest HPV-AF (133 [71·5%] of 186) and only two (1·5%) of 135 p16-positive patients were HPV-negative. Southern Europe (Spain) had the lowest HPV-AF (67 [7·82%] of 857) and the highest percentage of p16+/HPV– oropharyngeal cancer (28 [29·5%] of 95). Cohorts from northern and western Europe had intermediate proportions of p16+/HPV– patients with oropharyngeal cancer. North America was the region with the highest proportion of HPV-positive patients among p16-negative patients (18 [35·3%] of 51), half occurring in patients who were p16 equivocal (50–70% staining; 27 [52·9%] of 51; data not shown). Southern Europe was the region with the

lowest proportion of patients who were p16-negative and HPV-positive (32 [4·2%] of 762). More detailed information for each region and their cohorts are described in the appendix (pp 1–6).

For HPV testing, 214 (3%) of 7654 patients were tested by HPV RNA PCR (145 [68%] were p16 positive); 6194 (81%) by HPV DNA PCR (2832 [46%] were p16 positive); 976 (13%) by HPV RNA in-situ hybridisation (556 [57%] were p16 positive); and 1610 (21%) by HPV DNA in-situ hybridisation (1080 [67%] were p16 positive; appendix pp 10–11). p16 positivity was significantly lower with HPV DNA PCR than with other tests (p<0·0001; appendix pp 9–10) This p value is calculated comparing the proportion of p16+/HPV+ for each technique, but not included in any table. A specific HPV type was determined in 1137 (30·9%) of all 3679 HPV-positive patients: HPV16 single infection was the most prevalent type, found in 943 (92·2%) of 1023 patients with p16+/HPV+ oropharyngeal cancer with known HPV type detected, and in 97 (85·1%) of 114 patients with p16–/HPV+ oropharyngeal cancer with known HPV type detected (appendix p 7).

Unadjusted analyses of patient clinical and demographic characteristics according to the different combinations of p16 and HPV status are summarised in table 1. There were differences in age: double-positive patients were younger than discordant patients, who were in turn younger than double-negative patients (table 1). Differences were also observed in smoking and alcohol intake, with a much higher proportion of non-drinkers and never smokers in the double-positive subgroup than in any other group, and a greater proportion of ever smokers and alcohol drinkers in the double-negative subgroup than in any other group. There were also differences in stage according to the AJCC and UICC TNM 7 staging systems, with double-negative patients accounting for a higher proportion of patients with early disease than locally advanced disease (stage III–IVb). The rates of HPV negativity among p16-positive patients differed in those with tumours on the tonsil and base of the tongue to those in other subsites (p16+/HPV– in 310 [9·0%] of 3452 in tonsil and base of tongue *vs* 105 [29·7%] of 353 in other subsites, p<0·0001. The rate of p16–/HPV+ was 201 [8·4%] of 2407 in tonsil and base of tongue *vs* 88 [6·1%] of 1442 in other subsites, p<0·0001). Finally, there were also differences in treatment delivered, with a significantly greater proportion of double-negative patients than double-positive patients receiving surgery alone and palliative treatments (table 1).

We did a multinomial regression analysis to assess the adjusted determinants associated with the different positivity patterns, using double-negative oropharyngeal cancer as a reference, by each geographical region (appendix p 18). We stratified the analyses by region since there was an interaction with geographical regions (data not shown). Northern and western Europe reported

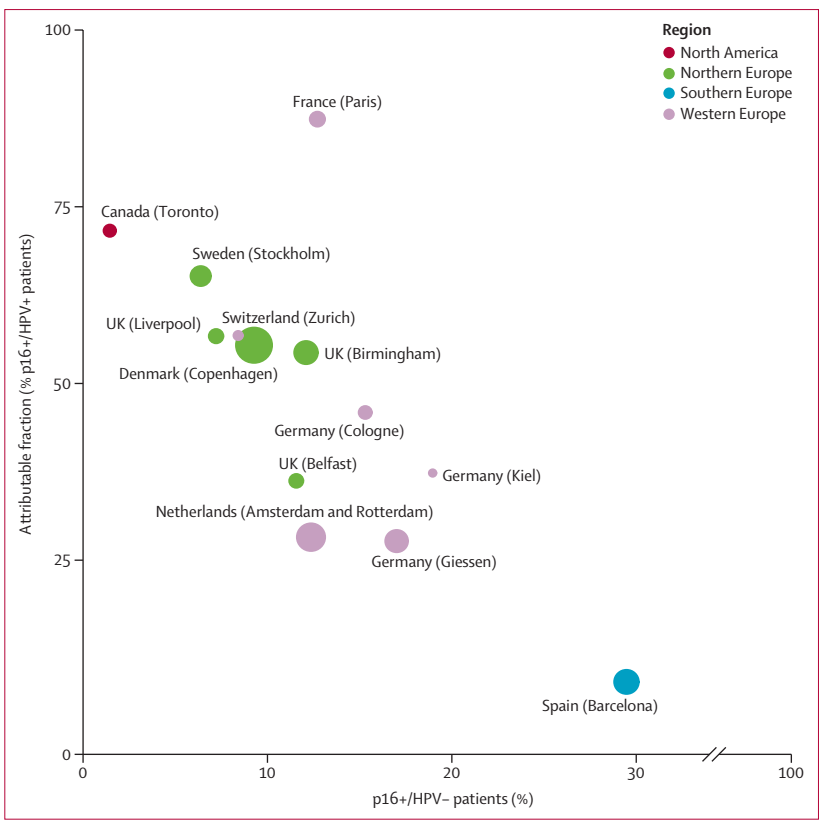


***Figure 2*: Proportions of p16-positive and HPV-negative patients versus p16-positive and HPV-positive patients according to the HPV-attributable fraction of each region**
The size of the circles is proportional to the number of cases in each cohort. *r*=–0·744, p=0·003. HPV=human papillomavirus.

a significant increase in double-positive patients since 2000–2004 (appendix p 18). This finding was not observed in southern Europe. Northern, western, and southern Europe showed similar trends to each other for alcohol and tobacco use, tumour subsite, and stage (appendix p 18). In northern and western Europe, double-positive patients were significantly more likely to be men than were double-negative patients, and patients tended to be younger in northern Europe than in other European regions. In North America, double-positive patients showed no significant differences compared with double-negative patients, except for less tobacco use (appendix p 18).

In southern Europe, p16+/HPV– patients had similar characteristics to double-negative patients, whereas in western Europe p16+/HPV– patients had more similarities to double-positive patients in smoking and drinking habits, tumour subsite, and period of diagnosis (appendix p 18). In northern Europe, there were fewer p16+/HPV– patients in the 67–100 year age group and more p16+/HPV– patients with TNM 7th edition stage than in other European regions. There were insufficient numbers in North America to draw conclusions (appendix p 18). In North America, discordant p16–/HPV+ patients had similar characteristics to double-negative patients.

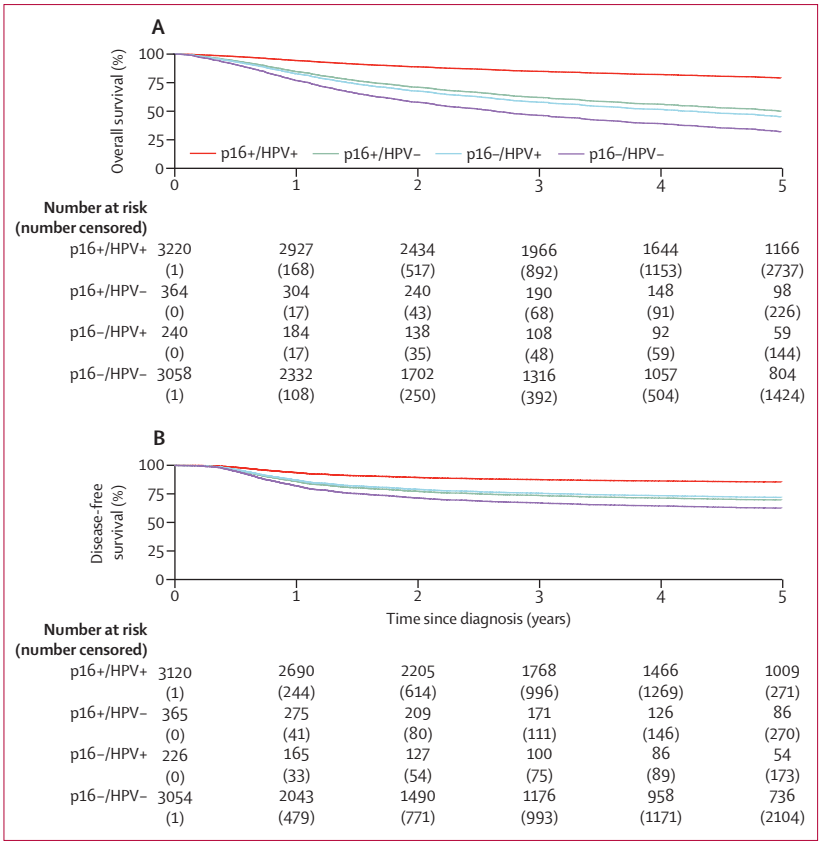


Figure 3: **5-year adjusted overall survival and 5-year adjusted disease-free survival for patients with oropharyngeal cancer by HPV biomarker positivity**
(A) Overall survival curve adjusted by biomarker combinations, sex, and cohort and stratified by treatment, stage (TNM 7th edition), age, smoking status, and year of diagnosis. (B) Disease-free survival curve adjusted by biomarker combinations, stage (TNM 7th edition), sex, year of diagnosis, and smoking status, and stratified by cohort and treatment. HPV=human papillomavirus.

	Deaths, n/ patients, N	Recurrences, n/ patients, N	Adjusted hazard ratio (95% CI)	p-value
Overall survival*				
All patients	2351/6882	NA	NA	<0·0001†
p16–/HPV–	1634/3058	NA	4·05 (3·59–4·58)	..
p16–/HPV+	96/240	NA	3·15 (2·50–3·97)	..
p16+/HPV–	138/364	NA	2·69 (2·21–3·29)	..
p16+/HPV+	483/3220	NA	Reference	..
Disease-free survival‡				
All patients	NA	1503/6765	NA	<0·0001†
p16–/HPV–	NA	950/3054	3·27 (2·84–3·76)	..
p16–/HPV+	NA	95/365	2·36 (1·87–3·97)	..
p16+/HPV–	NA	53/226	1·92 (1·42–2·60)	..
p16+/HPV+	NA	405/3120	Reference	..

NA=not applicable. *Hazard ratio adjusted for biomarker combinations, sex, and cohort, and stratified by treatment, stage, year of diagnosis (by group), and age (in quartiles). †Log-likelihood ratio test. ‡Hazard ratio adjusted for biomarker combinations, stage, sex, and year of diagnosis (by group) and stratified by cohort and treatment.

Table 2: **Overall survival and disease-free survival at 5 years by biomarker definitions of HPV status**

This situation was also observed for southern and western Europe, except for year of diagnosis, with more p16–/HPV+ patients with oropharyngeal cancer diagnosed in 2015 than in 2000 in both regions. There were fewer patients in the 60–66 years age group in Western Europe than in other European regions and fewer alcohol drinkers in southern Europe than in other European regions.

The overall median follow-up time was 5·1 years (95% CI 2·9–8·4). Overall survival was analysed in 6882 patients. The median overall survival was 6·9 years (95% CI 6·7–7·4), and was the highest in the cohorts from Paris, France (13·9 years, 95% CI 10·5–not reached [NA]) and Toronto, Canada (12·5 years, 10·7–NA), and the lowest in the cohort from Barcelona, Spain (3·5 years, 2·9–3·9; appendix pp 1–6). Disease-free survival was analysed in 6765 patients. Median disease-free survival was not reached in any cohort except for UK-Belfast (median disease-free survival 8·4 years (95% CI 6·0–NA). In total, there were 2896 deaths and 1554 recurrences during the study period.

Median overall survival was 15·0 years (95% CI 13·0–NA) for p16+/HPV+ cases, 3·5 years (3·2–3·8) for p16–/HPV– cases, 5·3 years (4·3–8·1) for p16–/HPV+ cases, and 6·7 years (5·0 –17·0) for p16+/HPV– cases (figure 3), but the median disease-free survival was not reached for any p16 and HPV combination.

5-year overall survival was 81·1% (95% CI 79·5–82·7) for p16+/HPV+ cases, 40·4% (38·6–42·4) for p16–/HPV– cases, 53·2% (46·6–60·8) for p16–/HPV+ cases, and 54·7% (49·2–60·9) for p16+/HPV– cases. 5-year disease free survival was 84·3% (95% CI 82·9–85·7) for p16+/HPV+ cases, 60·8% (58·8–62·9) for p16–/HPV– cases, 71·1% (64·7–78·2) for p16–/HPV+ cases, and 67·9% (62·5–73·7) for p16+/HPV– cases.

5-year adjusted hazard ratios (aHRs) for overall survival and disease-free survival are shown in table 2. Significantly lower overall survival and disease-free survival were seen in p16+/HPV– and p16–/HPV+ patients compared with p16+/HPV+ patients. For p16–/HPV+ patients, the aHR for overall survival was 3·15 (95% CI 2·50–3·97) and the aHR for disease-free survival was 2·36 (1·87–3·97). For p16+/HPV– patients, the aHR for overall survival was 2·69 (95% CI 2·21–3·29) and the aHR for disease-free survival was 1·92 (1·42–2·60). For p16–/HPV– patients (the patient cohort with the worst prognosis), the aHR for overall survival was 4·05 (95% CI 3·59–4·58) and the aHR for disease-free survival was 3·27 (2·84–3·76; table 2, figure 3).

These prognostic patterns were consistent regardless of region within Europe (figure 4, appendix p 13), anatomical subsite (figure 5; appendix pp 14–15), and when analysing HPV (PCR DNA, PCR RNA, HPV in-situ hybridisation DNA, RNA in-situ hybridisation) testing methods (appendix pp 19–23). Notably, there were insufficient numbers of discordant patients from North

America to draw conclusions in this cohort, especially for p16+/HPV– patients.

The results also remained unchanged when analyses were restricted to cases collected between 1999 and 2015 (appendix p 16) and when we excluded the cohorts from France and the Netherlands (data not shown).

Prognosis between the different p16 and HPV subgroups differed significantly by smoking status. Overall survival in p16+/HPV– never smokers was similar compared with double-positive never smokers (aHR of 1·53 (95% CI 0·82–2·87). However, p16+/HPV– ever smokers had a much worse prognosis than double-positive ever smokers, with an aHR of 2·94 (95% CI 2·37–3·64), but did not differ to that of either p16–/HPV+ ever smokers (aHR 3·13; 2·44–4·02) or double-negative patients ever smokers (4·06, 3·56–4·64; appendix p 12, 24). Differences by smoking status were also observed when stratifying by geographical region (appendix pp 13, 25–26), anatomical sub-site (appendix pp 14, 27–28) and HPV testing method (appendix pp 21–23).

Discussion

These findings, which are from the largest cohort (to our knowledge) of patients with oropharyngeal cancer, provide robust evidence that p16 and HPV discordance exists in these patients, with a prevalence that varies by geographical region, and that discordance between p16 and HPV biomarker status affects patient prognosis in terms of disease-free and overall survival. Moreover, our subgroup analyses show that the prognosis of patients with discordant p16+/HPV– oropharyngeal cancer depends on their smoking status. Never smokers have a significantly better prognosis than ever smokers, and their outcomes are similar to (but slightly worse than) p16+/HPV+ (double-positive) patients. p16+/HPV– patients who smoke have a significantly worse survival than p16+/HPV+ patients, with outcomes that are similar to (but slightly better than) p16–/HPV– patients. These results appear to be consistent regardless of geography, anatomical subsite, or HPV testing method and are consistent with previous findings from principal component analysis showing that a subgroup of patients with significantly worse survival and p16+/HPV– tumours clusters together with p16–/HPV– patients and that this subgroup is characterised by smoking, alcohol consumption, and a non-tonsillar location of the primary tumour.[19]

To the best of our knowledge, this multicentre, international study represents by far the largest effort to identify the contribution of p16 and HPV biomarkers in

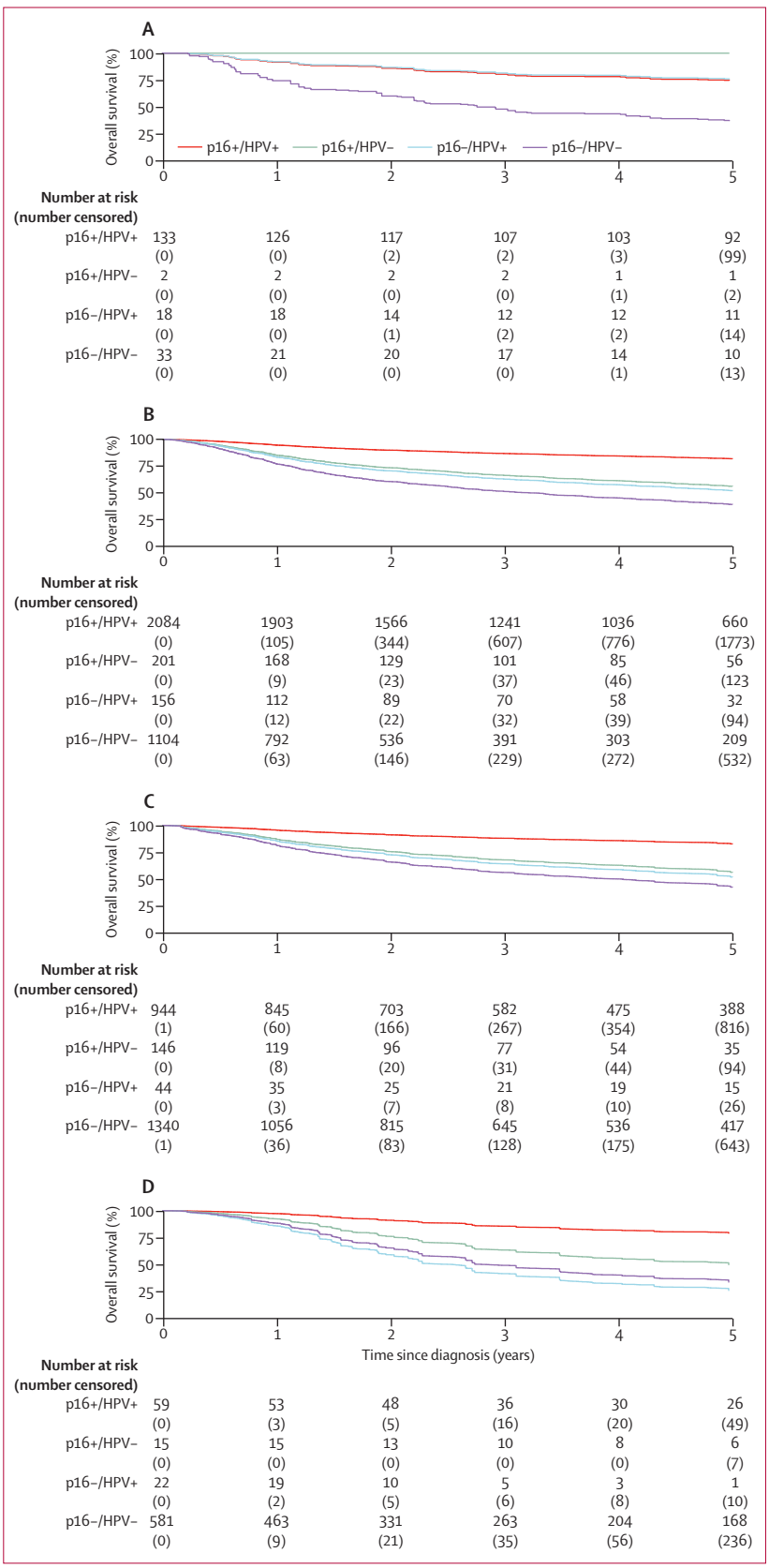
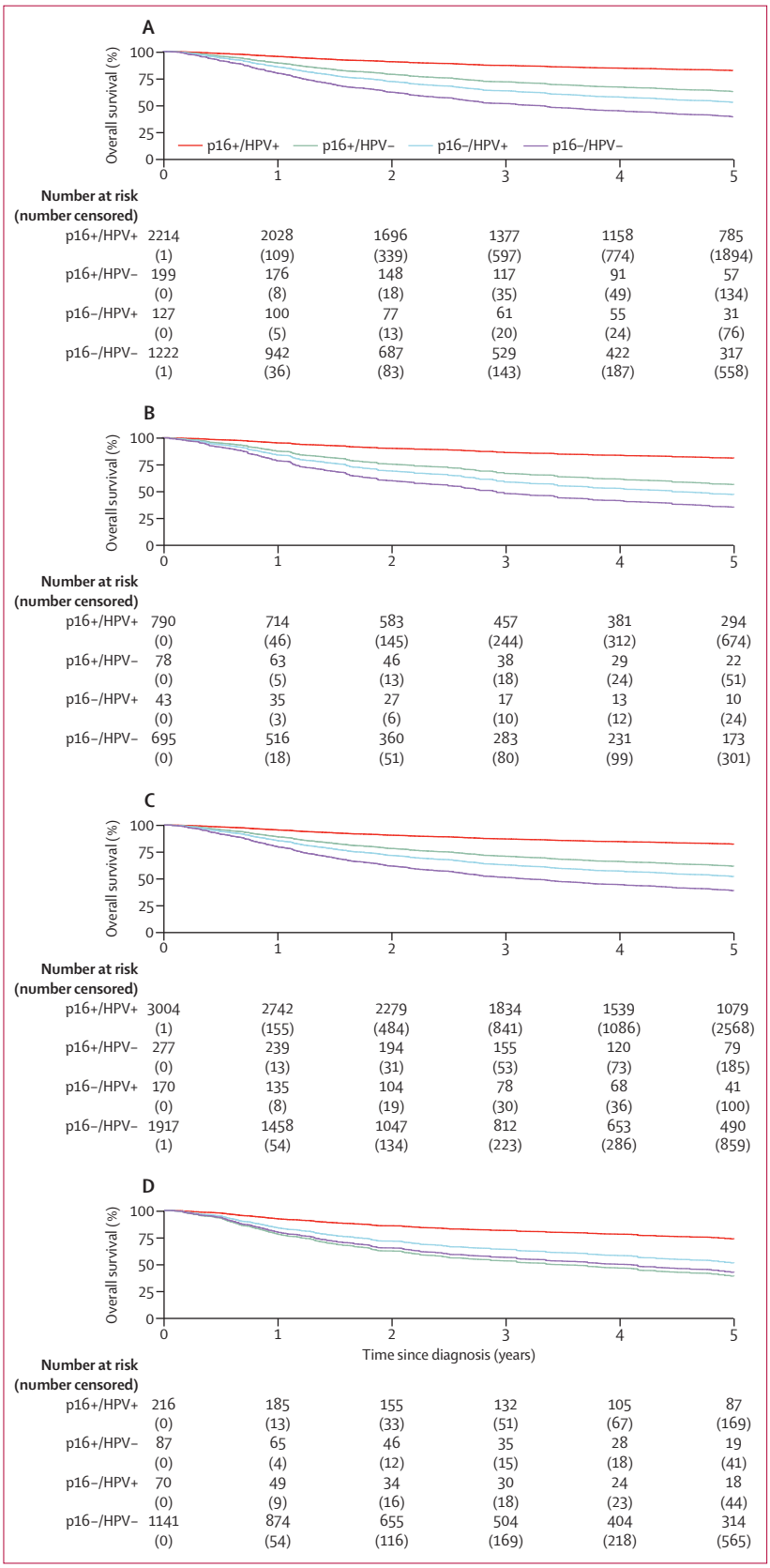


Figure 4: **5-year adjusted overall survival for patients with oropharyngeal cancer by HPV biomarker positivity and geographical region**
(A) North America. (B) Northern Europe. (C) Western Europe. (D) Southern Europe. Overall survival curves adjusted by biomarker combinations, sex, and cohort, and stratified by treatment, stage (TNM 7th edition), age, smoking status, and year of diagnosis. HPV=human papillomavirus.



determining prognosis in patients with oropharyngeal cancer. In an era where oropharyngeal cancer has been shown to have at least two subtypes with very different prognoses, this information is crucial to accurately classify this population of patients, and thereby aid appropriate patient selection for de-escalation or escalation of treatment. Our findings also have important implications when using the AJCC TNM staging system for prognosis, and for widely used practice guidelines such as the National Comprehensive Cancer Network guidelines, the College of American Pathologists Guidelines, and the American Society of Clinical Oncologists Guidelines, which currently recommend use of p16 immunohistochemistry for the purposes of oropharyngeal cancer classification, and for new and ongoing clinical trials that examine treatment de-escalation for patients with HPV-positive oropharyngeal cancer.[8]

Different p16+/HPV− oropharyngeal cancer tumours appear to overexpress p16 due to different mechanisms. Patients with p16+/HPV− tumours who do not smoke might mostly have HPV-mediated tumours, but possibly at lower copy numbers than all p16+/HPV+ patients, and therefore can only be detected by techniques that have the highest sensitivity, such as HPV RNA PCR. These could also relate to the group of so-called copy number silent tumours, a potentially separate genetic subgroup of HPV-negative tumours with a more favourable prognosis.

However, in patients who smoke, worse outcomes are in part driven by an increase in cancer-related deaths (as seen from the worse disease-free survival results), and not simply by an increase in deaths from non-cancer, smoking-related comorbidities. In these tumours, p16 expression might be due to causes that are not related to HPV, but to other molecular causes such as genomic alterations of genes active in the retinoblastoma protein pathway.[25] This mis-classification could have substantial implications for counselling, treatment decision making, and outcomes. Because these discordant patients have significantly worse outcomes than true HPV-mediated oropharyngeal cancer, it would not be appropriate to routinely consider treatment de-escalation for these patients. Indeed, in view of their poorer prognosis, these patients could in fact be candidates for clinical trials evaluating escalation of treatment. Based on our data, if p16 immunohistochemistry is used alone to determine

Figure 5: **5-year adjusted overall survival for patients with oropharyngeal cancer by HPV biomarker positivity and oropharyngeal subsite**
(A) Tonsil; adjusted by biomarker combinations and sex, and stratified by treatment, stage (TNM 7th edition), age quartile, smoking status, and year of diagnosis. (B) Base of the tongue; adjusted by biomarker combinations, age at diagnosis, year of diagnosis, stage (TNM 7th edition), and cohort, and stratified by treatment and smoking status. (C) Tonsil and base of the tongue; adjusted by biomarker combinations and sex, and stratified by treatment, stage (TNM 7th edition), age quartile, year of diagnosis, smoking status, and cohort. (D) Other oropharyngeal subsites adjusted by biomarker combinations, sex, and year of diagnosis, and stratified by treatment, stage (TNM 7th edition), age quartile, and geographical regions. HPV=human papillomavirus.

HPV mediation, 8·1% of p16-positive patients worldwide, and up to almost 26% in regions of low HPV-AF such as southern Europe are HPV-negative ever smoker patients and thus would be incorrectly classified as having HPV-related tumours.

Discordant p16–/HPV+ patients showed significantly worse recurrence rates, survival, and prognosis than did p16+/HPV+ patients, with similar outcomes to (if slightly better than) p16–/HPV– patients, regardless of smoking status, geographical location, anatomical site, or HPV testing method. However, p16–/HPV+ patients should not be simply treated as p16–/HPV– patients. Their prognosis seems to be somewhat better than the double-negative patients, and practitioners need to question whether escalation regimens would be appropriate or different to those used for double-negative patients. More in-depth molecular research is required to investigate the mechanism of disease in these tumours.

Most de-escalation clinical trials determine risk stratification and eligibility based on p16 immunohistochemistry positivity alone, thereby introducing potential bias to the results of the studies. Furthermore, this approach might pose harm to the portion of their recruited patients with oropharyngeal cancer who are ever smokers and who have p16-positive tumours that are not actually HPV driven, and hence have a worse prognosis. Dual testing with p16 immunohistochemistry and an HPV DNA or RNA test should therefore be implemented as standard in trials that assess de-escalation strategies. In the future, other tests for confirmation of HPV relatedness might be available for use in clinical practice. For example, several cell-free HPV DNA assays have been developed, and some are already available commercially.

Our study also showed that the p16 and HPV discordant rates vary across geographical regions, depending on the HPV-AF in that region, regardless of the HPV test method used. Discordant oropharyngeal cancer rates are high in areas with lower HPV-AF, such as southern Europe. On the other hand, North America had the highest HPV-AF and the lowest percentage of p16+/HPV– patients. Northern Europe had intermediate HPV-AFs and p16+/HPV– rates. The reasons for these variations are unknown, but might be due in part to a learning curve for p16 staining and scoring and inherent inter-laboratory and inter-rater variability. The rate of discordant patients could also be related to the prevalence of other risk factors. For example, in a population of patients who smoked more, there would be a greater probability that p16 is inactivated by mutation or promoter methylation. Other influences on tumour evolution that reduce the driving role of viral oncogenes are also conceivable. Our study did not include samples from Asia, South America, or Africa, and therefore we cannot be definitive about the generalisability of our findings in those regions. However, studies show that incidence rates of HPV-mediated oropharyngeal cancer are low in Asia,[26–28] South America,[27,28] and Africa,[27,28] which suggests that the situation in these regions might be similar to that in southern Europe.

Although the most accurate and safest testing regimen would be to do p16 immunostaining combined with or followed by HPV DNA or RNA testing, our findings also suggest that there might be a role for tailoring recommendations for clinical practice to specific regions, considering whether dual testing would be cost-effective in areas with high prevalence and low discordance such as Canada.

Similar to previous reports,[29] this study also showed that the prevalence of p16+/HPV– in non-palatine or lingual-tonsil primary was much higher than in tonsil and base of tongue subsites. Therefore, for a p16-positive oropharyngeal cancer arising from non-palatine or lingual tonsil, confirmatory HPV testing is particularly recommended. However, one must also bear in mind that previous data has shown that neither HPV positivity nor p16 positivity, either alone or in combination, seemed to correlate with survival in non-palatine or lingual tonsil cases.[29]

Our study has several limitations. The retrospective nature of our cohort might have hampered the accurate characterisation of some patient risk factors, such as tobacco and alcohol use, since this kind of information could only be obtained from medical records from the institutions involved in the study. Most participating institutions tested all samples for both p16 immunohistochemistry and HPV-DNA, but the cohort from France and the Netherlands (representing 3·6% of the included patients) only tested samples for HPV-DNA if they were positive for p16. In the cohort from North America, only two patients were p16+/HPV– and both these patients had an excellent survival, so few conclusions can be made for this subgroup in this region.

In addition, various antibodies were used for the p16 immunohistochemistry assay, and HPV evaluation was done by assessing either DNA or RNA and with various techniques (ie, PCR or in-situ hybridisation) depending on the institution preference. A meta-analysis[28] showed that p16 immunohistochemistry was more consistent with HPV tested by in-situ hybridisation than with PCR. However, studies with very sensitive PCR assays were included in that meta-analysis, which probably overestimates HPV positivity rates and might have impacted the results and conclusions. The College of American Pathologists' guidelines state that there are not sufficient data to recommend one p16 antibody, platform, or set of test conditions over another.[30]

The heterogeneity in test assays, techniques, and scorers could also be considered a strength of this study, because it replicates the real-world setting, in which different institutions would use different p16 and HPV assays. An alternative approach would have been to undertake a centralised re-analysis of all the samples

using the same p16 and HPV assays in one laboratory. Apart from being not logistically feasible, such a centralised re-analysis would not reflect the real world situation, and therefore we believe that it would have less application clinically. Despite the variability in test performance that our study design might impart, the study results show large differences in outcomes between the different p16/HPV subgroups, which are consistently seen across different geographical regions and HPV different testing methods, strongly suggesting that these are real and robust differences, and not simply due to different testing assays, methods, or laboratory practices.

Our study also has several other strengths. The characterisation of this subgroup of patients, particularly p16+/HPV–, requires a large sample size. Our study provides an explanation for the contradictory results from studies that assess patients with discordant oropharyngeal cancer, which have mainly included patients from single institutions and have not been sufficiently powered to characterise the smaller numbers of patients with discordant p16 and HPV oropharyngeal cancer, especially with regard to local and regional differences in smoking status and AFs, resulting in widely varying reported rates. Furthermore, no geographical comparisons were possible until now.[18,19] To the best of our knowledge, this study represents the largest sample of patients with discordant p16 and HPV oropharyngeal cancer, and has enabled us to characterise this group of patients adequately.

Our findings indicate that classification of patients with oropharyngeal cancer based on p16-positive immunohistochemistry alone is inadequate in a trial setting, and is likely to be insufficient in routine clinical practice, both for predicting prognosis and when selecting treatment. Routine HPV testing alongside p16 evaluation, or at least following a positive result on p16 immunohistochemistry, should be mandated in oropharyngeal cancer clinical trials. It is also recommended in the clinical setting for more accurate counselling on prognosis, and in future circumstances in which treatment de-escalation or intensification are being considered. This approach is particularly important in patients with oropharyngeal cancer who smoke, and in those geographical regions with low HPV-AFs.

Contributors

HMe, LA, and MT devised the idea for the study. LA and ST had access to and verified the data and were responsible for data analysis. All authors contributed to study design, data collection, interpretation, and drafting of the manuscript. The corresponding authors had full access to all the data in the study. HMe and LA had final responsibility for the decision to submit for publication.

Declaration of interests

HMe reports grants from UK National Institute of Health research, Cancer Research UK, the UK Medical Research Council, and AstraZeneca; advisory board fees from AstraZeneca, MSD, Merck, Nanobiotix, and Seagen; and is Director of Warwickshire head neck clinic and Docpsert Health. CRL reports a grant from the Dutch Cancer society, consulting fees from Merck & Co, and expenses from the European Head and Neck Society. JPK reports grant funding from MSD (funding for the project Implementation of a German Oropharyngeal Cancer Registry Providing State-of-the-Art-HPV-Diagnostics). Data derived from this project were used in the present study. The funders had no influence on the design of the study, the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to publish the results. JPK also declares research grant funding from the European fund for regional development (EFRE) for ImmunPredict (FKZ EFRE EFRE-0801308). LM reports payment to institution from 20200059/Stockholms Läns Landsting. NW reports a grant from Merk, Sharpe, & Dohme (MSD; for the project Implementation of a German Oropharyngeal Cancer Registry Providing State-of-the-Art-HPV-Diagnostics). Data derived from this project were used in the present study. The funders had no influence on the design of the study, the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to publish the results. LM is also supported by the Cologne Clinician Scientist Programme, funded by the German Research Council (FI 773/15-1; 50% salary funding for the project Monitoring disease status in patients with oropharyngeal squamous cell carcinoma by detection of cell-free human papillomavirus DNA in liquid biopsies); funding from EFRE (salary funding for the project ImmunPredict FKZ EFRE EFRE-0801308); honoraria for lectures from MSD, and has a patent for CRISPR-based HPV-diagnostics (EP 22197523.8). RHB declares grants from the Dutch Cancer Society to institution; from ZonMW Government to institution; GenMab BV to institution; consulting fees from Nanobiotix; and payment from the Italian Association for Cancer Research as an unpaid board member. SW declares a grant from MSD under the administration of the University of Cologne (for the project Implementation of a German Oropharyngeal Cancer Registry Providing State-of-the-Art-HPV-Diagnostics). Data derived from this project were used in the present study. The funders had no influence on the design of the study, the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to publish the results. TD reports a research grant from the Swedish Cancer Foundation. ST reports grants from Merck, Roche, GlaxoSmithKline, Vitro, Hologic, and Seagene and consulting fees from Merck. All other authors declare no other competing interests. HMe is a National Institute for Health Research (NIHR) Senior Investigator. The views expressed in this article are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

Data sharing

Deidentified participant data is available to share on request to the corresponding author, following completion of data sharing agreements, starting from Jan 1, 2025. The study protocol and statistical analysis plan will also be provided.

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