Human papillomavirus (HPV) and rising oropharyngeal cancer incidence in the United States

Chaturvedi, et al

Supplementary Statistical Appendix

Incidence projections: We projected the future burden of oropharyngeal, cervical, and other head and neck cancers through the year 2030 using observed age-specific incidence trends from nine cancer registries within the SEER program in age-period-cohort models [18-20]. Using 5-year calendar periods (1973-2007) and 5-year age groups (30-84 years), we estimated the fitted age-at-onset curves for each of 17 overlapping 5-year birth cohorts (1893-1898, 1898-1903, 1903-1908,.....1968-1973, denoted by the mid-point of the interval). For birth cohorts already under observation, incidence rates were extrapolated using cohort-specific age-at-onset curves. For future birth cohorts, incidence rates were projected on the basis of a fitted age-at-onset curve whose relative level compared to observed cohorts was extrapolated on the basis of the observed net drift, which captures incidence trends across both birth cohorts and calendar periods[18]. Bootstrap 95% prediction intervals were constructed that reflected the estimated uncertainty of the parameters and the expected variability of future cohort and period deviations, which was estimated from the observed variability.

Incidence projections assume that the current observed increases in incidence for oropharyngeal cancers and decreases in incidence for cervical and other head and neck cancers would continue into the future. We believe these assumptions represent a plausible scenario given continued increases in oropharyngeal cancer incidence rates from aging of current birth cohorts, decreases in cervical cancer incidence from screening, and decreases in incidence of other head and neck cancers from recent reductions in tobacco use in the U.S.

Supplementary material

Supplementary Table 1 shows population characteristics, oropharyngeal cancer incidence rates and trends for the three registries that participate in SEER's Residual Tissue Repository (RTR) Program and seven other registries included in the SEER9 Program.

Supplementary Table 2 shows the prevalence of HPV infection in 69 cervical cancer samples which were used as positive controls in the study. Prevalence of HPV by the Inno-LiPA assay was estimated for all evaluable cervical cancer specimens, while the estimation of prevalence by the HPV16 viral load, HPV16 E6/E7 mRNA, and HPV16 *in situ* hybridization assays was restricted to Inno-LiPA HPV16 positive specimens.

Supplementary Table 3 shows agreement between HPV16 assays—Inno-LiPA, viral load, E6/E7 mRNA, and *in situ* hybridization—among oropharyngeal cancer cases. Agreement was assessed using the kappa statistic.

Supplementary Table 4 shows comparisons of HPV prevalence in oropharyngeal tumors (1984-2004) by age, sex, and race. Results are shown for HPV detection by Inno-LiPA, HPV16 viral load, HPV16 E6/E7 mRNA, and HPV16 *in situ* hybridization.

Supplementary Table 5: Predictors of overall survival included in the multivariate model for comparisons of overall survival between HPV-positive (determined by Inno-LiPA) and HPV-negative oropharyngeal cancer cases.

Supplementary Figure 1 shows the comparison of HPV16 viral load (panel A) and HPV16 E6/E7 oncogene transcript levels (Panel B) between Inno-LiPA HPV16 positive oropharyngeal cancers and cervical cancers. Comparisons were conducted using the non-parametric Wilcoxon rank-sum test. The horizontal lines in the figures represent median values.

Supplementary Figure 2: Trends in HPV prevalence as determined by the Inno-LiPA assay across four calendar periods (1984-1989, 1990-1994, 1995-1999, and 2000-2004) are shown for age groups (panel A); gender (panel B); and race (panel C). P-values for trend across calendar periods within each demographic subgroup were evaluated using the Cochran-Armitage trend test.

Supplementary Figure 3 complements the **Figure 2 Panel A** presented in the main text. The figures show Kaplan-Meier survival curves and 95% confidence intervals for HPV-positive and HPV-negative oropharyngeal cancer cases, as determined by the HPV16 E6/E7 mRNA assay (Panel A); HPV16 viral load assay (Panel B); and HPV16 *in situ* hybridization assay (Panel C).

Supplementary Figure 4 shows hazard ratios (HRs) and 95% CIs for comparisons of overall survival between HPV-positive oropharyngeal cancer cases (determined by Inno-LiPA, HPV16 viral load, HPV16 E6/E7 mRNA expression, and HPV16 *in situ* hybridization) and HPV-negative cases for the respective assay. HRs were adjusted for age (<50, 50-59 years, 60-69 years, and 70+ years), sex, race (white, black, and other races), calendar period (1984-1989, 1990-1994, 1995-1999, and 2000-2004, treated as a 1 degree-of-freedom ordinal variable), SEER registry, stage (localized, regional, and distant), radiotherapy, chemotherapy, and surgery.

Supplementary Figure 5 shows prevalence of p16 in oropharyngeal tumors across calendar time (**Panel A**). Observed prevalence was not corrected for potential loss in assay sensitivity because of evidence of low assay sensitivity for the older calendar periods in cervical cancer positive controls (prevalence in 69 cervical cancers= 9.1% during 1984-1989; 60.0% during 1990-1994; 73.7% during 1995-1999; 77.9% during 2000-2004). **Panel B** shows the Kaplan-Meier estimates and 95% CIs for overall survival for p16-positive and p16-negative oropharyngeal cancer cases (log-rank p-value<0.001).

Supplementary Table 1: Comparison of population characteristics and oropharyngeal cancer incidence between Iowa, Hawaii, and Los Angeles and seven other registries included in the SEER9 Program, 1984-2004

	Hawaii, Iowa, and Los Angeles n (%)	Other registries in SEER9 [a] n (%)
Age, years		
<50	72,005,560 (56.4)	137,264,734 (56.9)
50-59	22,816,166 (17.8)	43,520,946 (18.1)
60-69	16,984,348 (13.3)	31,384,832 (13.0)
70+	16,034,522 (12.5)	28,841,658 12.0)
Gender		
Male	61,733,185 (48.3)	115,755,293 (48.0)
Female	66,107,411 (51.7)	125,256,877 (52.0)
Race		
White	95,511,376 (74.7)	196,373,137 (81.5)
Black	9,036,943 (7.1)	27,765,025 (11.5)
Other	23,292,277 (18.2)	16,874,008 (7.0)
Oropharyngeal cancer incidence, 1988-2004		
rate per 100,000 (95% CI)	3.0 (2.9-3.1)	3.7 (3.6-3.8)
Trend in oropharyngeal cancer incidence, 1988-2004		
annual percent change (95% CI)	3.5 (1.4-5.6)	2.9 (1.1-4.6)

[a] Registries include: Atlanta, Connecticut, Detroit, New Mexico, San Francisco-Oakland, Seattle, and Utah.

Supplementary Table 2: Sensitivity of HPV assays on cervical cancers across calendar periods [a]

	Inno-LiPA any HPV	HPV16 viral load	HPV16 E6/E7 mRNA expression	HPV16 ISH
1984-1989				
n tested	11	5	5	5
n evaluable [b]	10	5	4	5
% positive	80.0	100.0	100.0	80.0
1990-1994				
n tested	20	12	12	12
n evaluable [b]	19	12	9	12
% positive	100.0	66.7	88.9	75.0
1995-1999				
n tested	20	13	13	13
n evaluable [b]	19	13	13	13
% positive	89.5	84.6	92.3	84.6
2000-2004				
n tested	18	10	10	10
n evaluable [b]	18	10	8	10
% positive	83.3	60.0	87.5	70.0

HPV= Human papillomavirus.

LiPA= Linear probe assay.

mRNA= Messenger RNA.

ISH= In situ hybridization.

[a] Sensitivity was assessed using 69 cervical cancers as positive controls, given HPV's role as a necessary cause. Sensitivity of the Inno-LiPA assay was assessed using all 69 cervical cancers. Calculation of sensitivities for the HPV16 viral load, E6/E7 mRNA expression, and *in situ* hybridization assays was restricted to 40 samples positive for HPV16 on the Inno-LiPA assay.

[b] Evaluability of specimens for the Inno-LiPA and HPV16 viral load assays was determined based on the number of copies of human endogenous retrovirus-3 (ERV-3). Evaluability of specimens for the HPV16 E6/E7 mRNA assay was determined using the number of copies of human ribosomal protein large P0 (RPLPO). Specimens with ≥ 3 copies of ERV-3 or RPLPO were considered as evaluable. All specimens were evaluable for the HPV16 ISH assay.

Supplementary Table 3: Agreement between laboratory assays for HPV16 status in oropharyngeal cancers

	HPV16 viral load **kappa (95% CI)**	**HPV16 E6/E7 mRNA expression** **kappa (95% CI)**	**HPV16 ISH** **kappa (95% CI)**
Inno-LiPA HPV16	0.79 (0.71-0.86)	0.85 (0.77-0.92)	0.67 (0.58-0.76)
HPV16 viral load		0.95 (0.90-0.99)	0.76 (0.67-0.85)
HPV16 E6/E7 mRNA expression			0.79 (0.70-0.88)

HPV= Human papillomavirus.

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Supplementary Table 4: Comparison of HPV prevalence in oropharyngeal cancers across demographic subgroups (1984-2004)

Characteristic	Inno-LiPA % positive	Chi-square p-value	HPV16 viral load % positive	Chi-square p-value	HPV16 E6/E7 mRNA expression % positive	Chi-square p-value	HPV16 ISH % positive	Chi-square p-value
Age, years		<0.001		<0.001		<0.001		<0.001
<50	77.5		59.2		62.8		54.9	
50-59	49.4		35.8		42.9		33.7	
60-69	30.3		18.4		20.3		16.7	
70+	26.3		15.8		15.9		11.9	
Gender		0.03		0.007		0.001		<0.001
Male	47.4		34.6		40.1		32.7	
Female	30.8		15.4		12.8		9.3	
Race		0.003		0.005		0.001		0.08
White	47.6		34.8		40.0		29.2	
Black	18.9		8.1		6.2		13.5	
Other	50.0		33.9		40.8		33.3	

HPV= Human papillomavirus.

LiPA= Linear probe assay.

mRNA= Messenger RNA.

ISH= In situ hybridization.

Supplementary Table 5: Predictors of overall survival of oropharyngeal cancer cases (n=271)

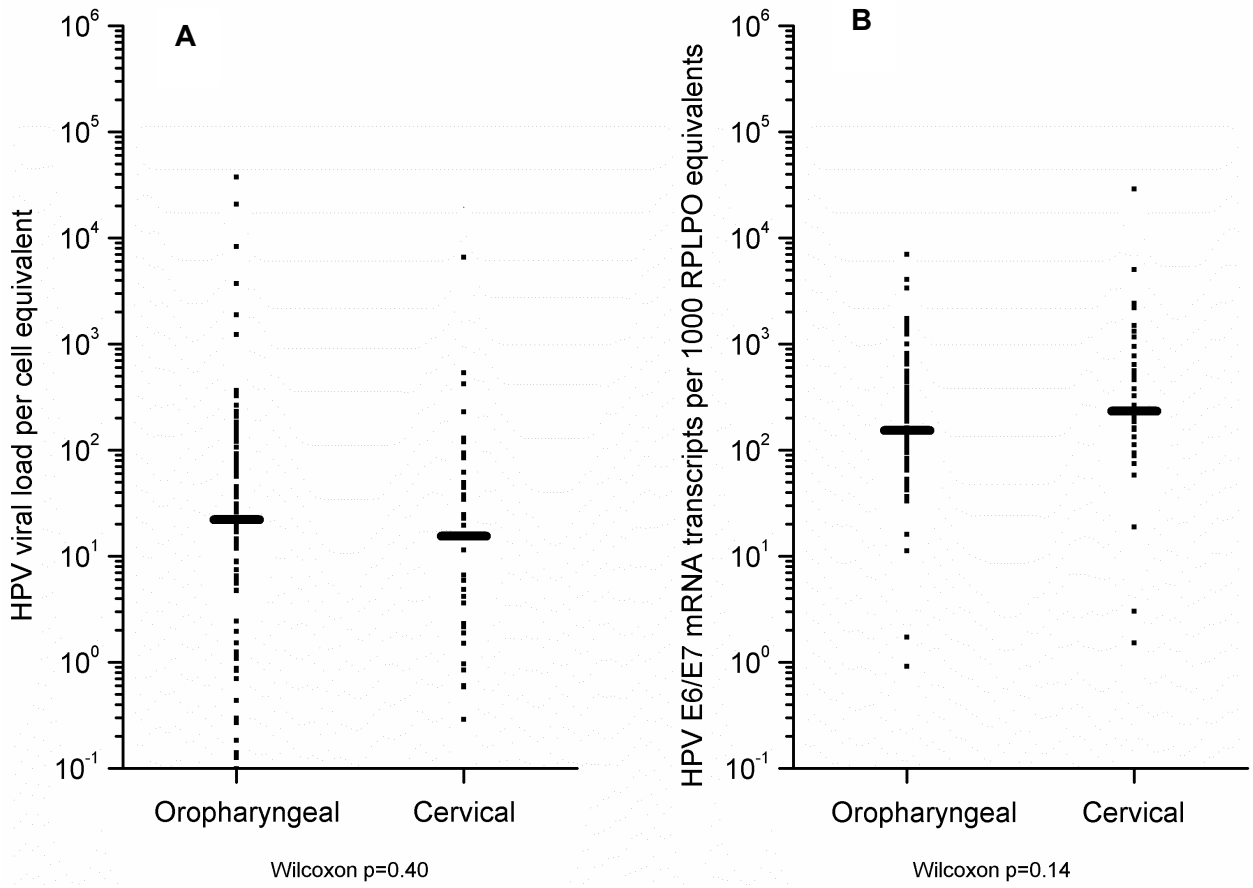
Characteristic	Adjusted HR (95% CI) [a]
Age, years	
<50	1.0
50-59	0.83 (0.49-1.42)
60-69	0.98 (0.57-1.66)
70+	1.70 (0.98-2.95)
Gender	
Female	1.0
Male	0.95 (0.64-1.39)
Race	
White	1.0
Black	1.04 (0.65-1.66)
Other	0.79 (0.51-1.22)
Registry	
Hawaii	1.0
Iowa	1.41 (0.77-2.58)
Los Angeles	1.11 (0.73-1.70)
Stage	
Localized	1.0
Regional	1.91 (1.29-2.85)
Distant	2.40 (1.36-4.22)
Radiotherapy [b]	
No	1.0
Yes	0.65 (0.43-0.98)
Surgery [b]	
No	1.0
Yes	0.51 (0.36-0.74)
Chemotherapy [b]	
No	1.0
Yes	1.90 (1.28-2.83)

[a] Hazard ratios from Cox proportional hazard regression models were adjusted for all variables in the table and additionally for tumor HPV status (determined by Inno-LiPA) and calendar period (1984-1989, 1990-1994, 1995-1999, and 2000-2004, modeled as an ordinal variable).

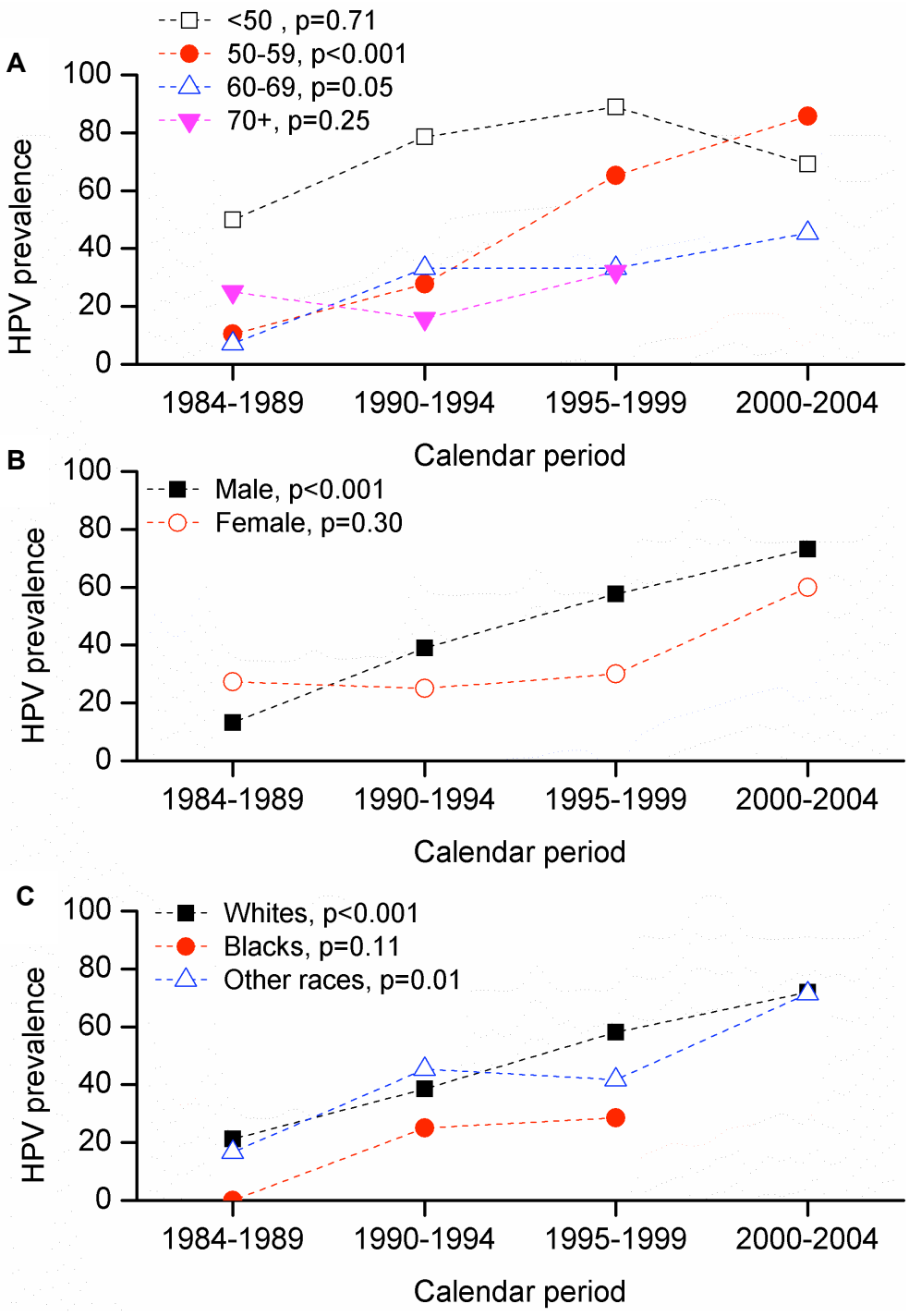
[b] Indicate the use of radiotherapy, surgery, or chemotherapy as the first course of cancer-directed therapy. In univariable analyses across the clinical variables (stage, radiotherapy, surgery, and chemotherapy), receipt of radiotherapy was significantly associated with receipt of

chemotherapy, and regional and distant stage; receipt of surgery was significantly associated with lack of chemotherapy; and receipt of chemotherapy was significantly associated with regional and distant stage. Tumor HPV status was significantly associated with regional and distant stage, but not associated with receipt of radiotherapy, chemotherapy, or surgery.

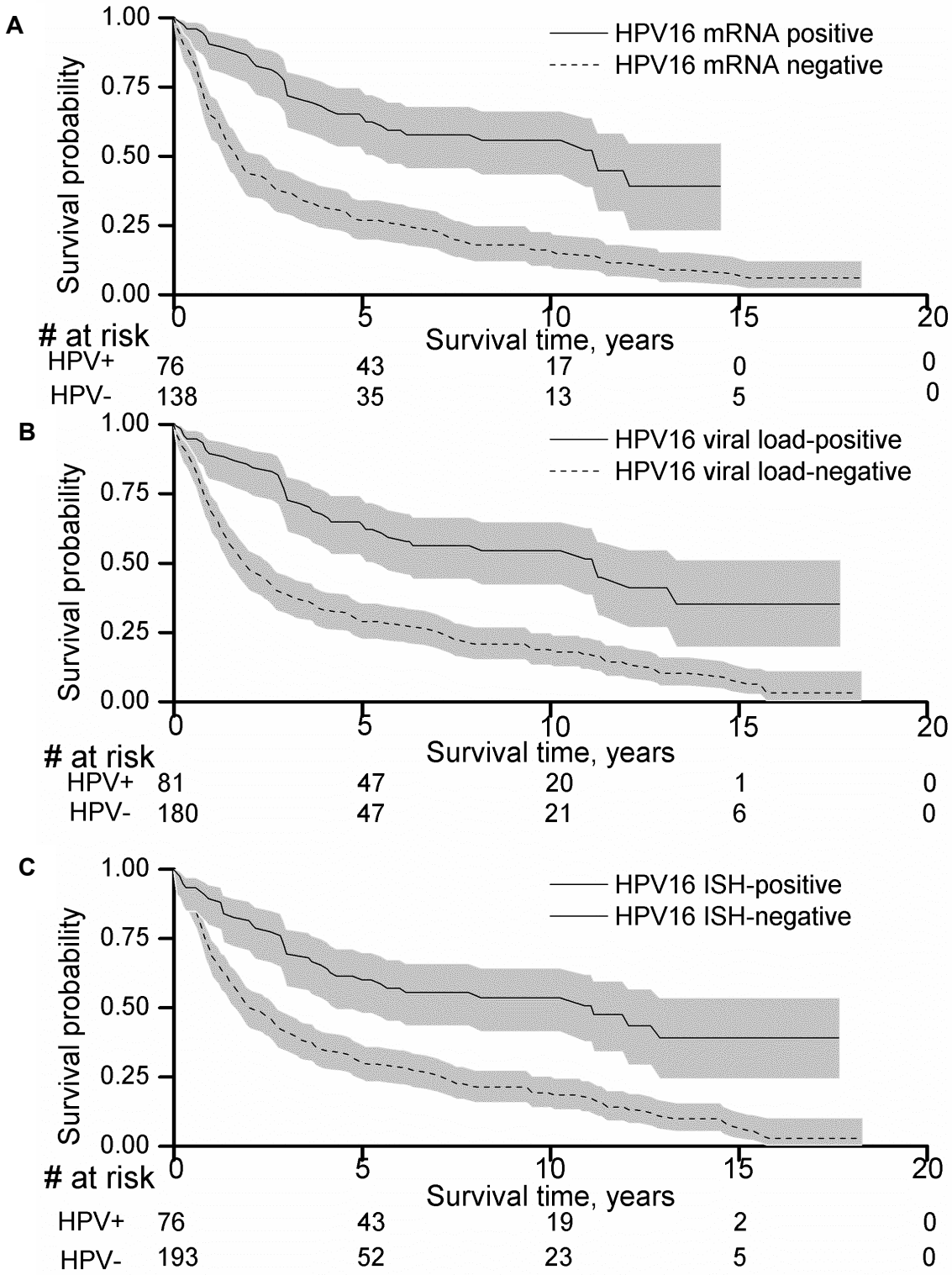
Supplementary Figure 1: Comparison of HPV16 viral load and E6/E7 mRNA levels between oropharyngeal cancers and cervical cancers



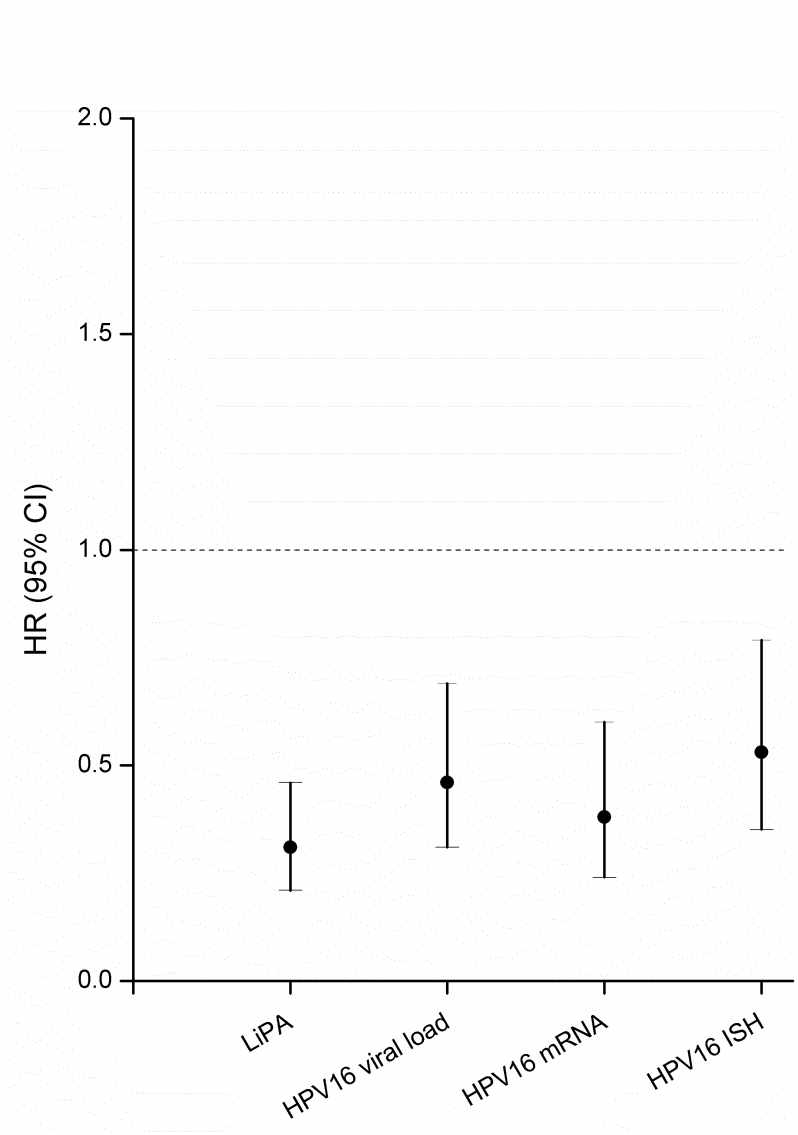
Supplementary Figure 2: Trends in prevalence of HPV infection in oropharyngeal cancers across calendar time among demographic subgroups



Supplementary Figure 3: HPV16 infection and long-term overall survival of oropharyngeal cancer cases



Supplementary Figure 4: Association of HPV infection with overall survival of oropharyngeal cancer cases



Supplementary Figure 5: Prevalence of p16 in oropharyngeal cancers across calendar periods and association of p16 status with overall survival