Human papillomavirus in oropharyngeal squamous cell carcinoma in Iceland

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Oropharyngeal squamous cell carcinoma tissue from patients in Iceland was tested for HPV infection using $p16^{INK4a}$ immunohistochemistry and polymerase chain reaction (PCR).

The tissue and the corresponding health records are from the only hospital in Iceland to treat oropharyngeal cancer during the observed period. Of 89 patients, about half of the patients are aged 50 to 70 at diagnosis. All have meaningful p16 coloring results. All patients diagnosed in the 21-year period 1994 to 2014 were included in this study. Survival is based on medical records from August 2019. 70 have meaningful polymerase chain reaction results. Survival is compared between HPV-positive and HPV-negative patients and by gender.

1 Incidence

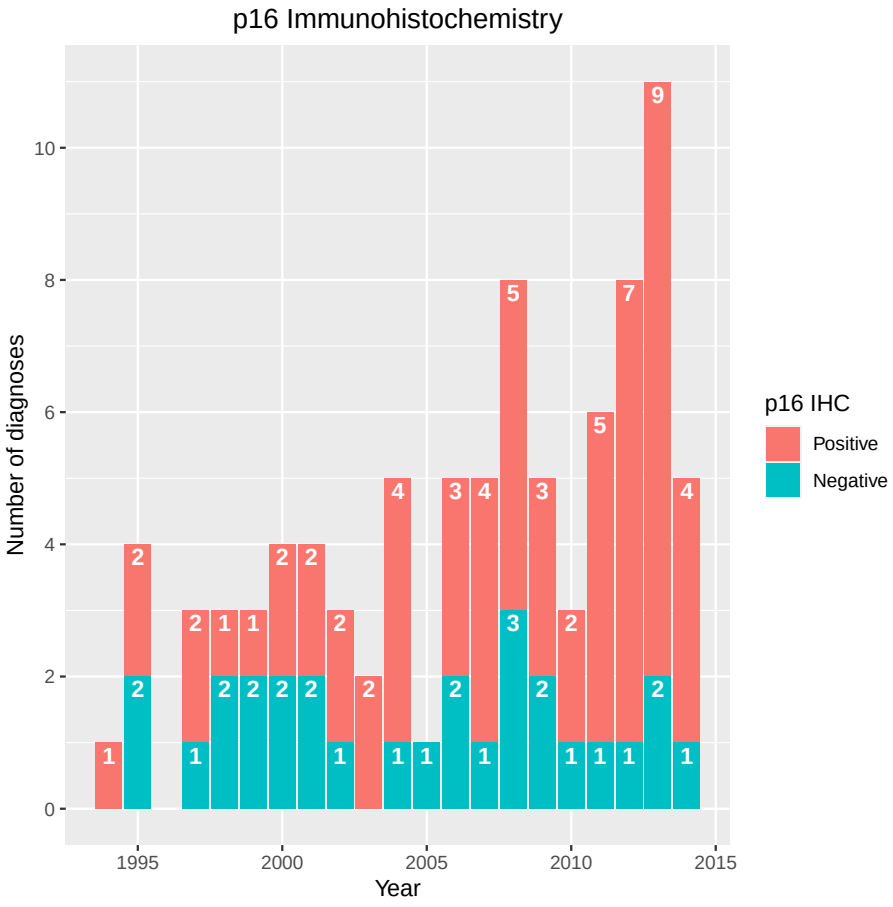


Figure 1: OPSCC incidence by p16 stain result

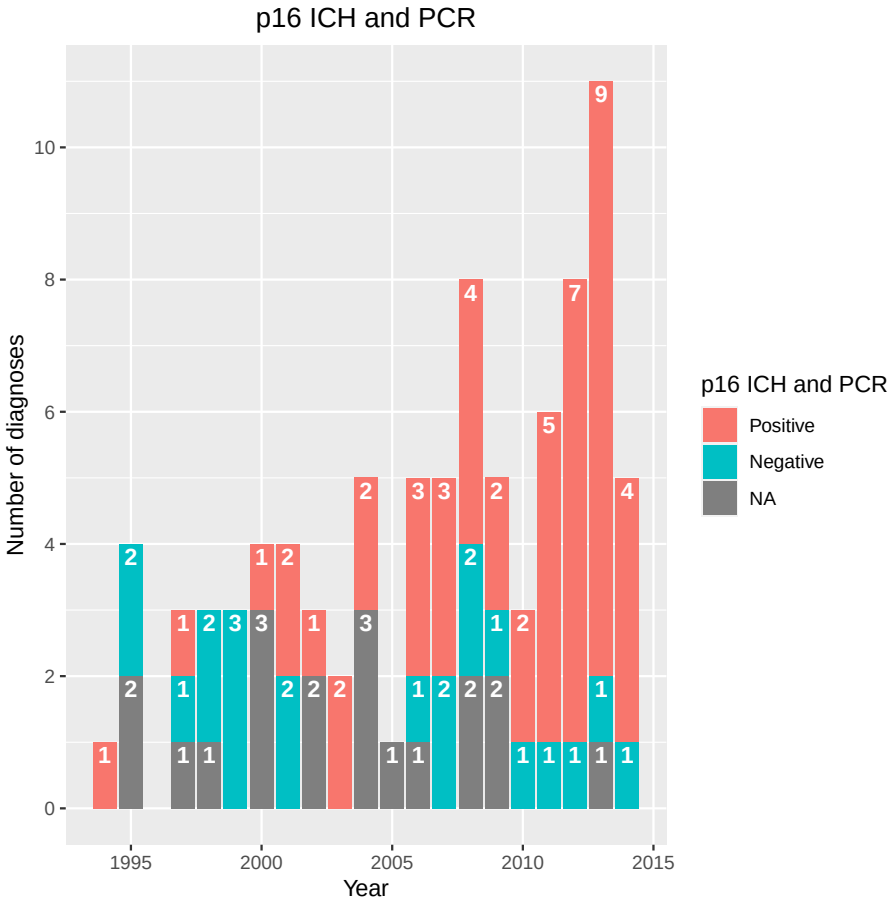


Figure 2: OPSCC incidence by combined p16 stain and PCR results

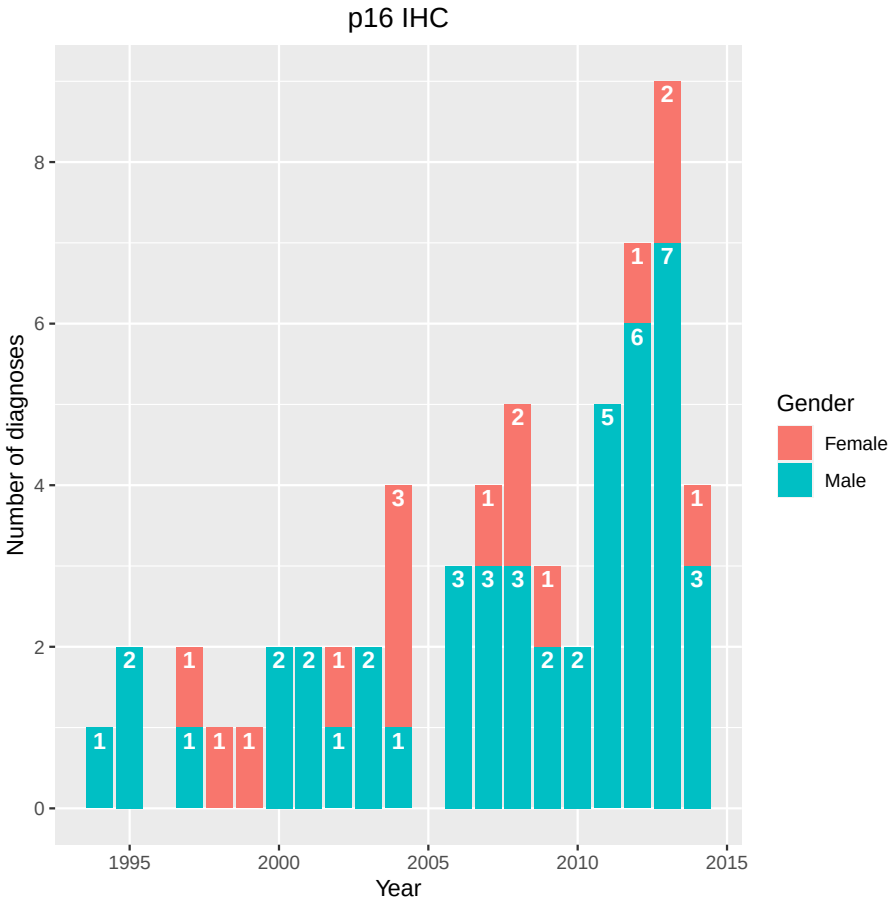


Figure 3: OPSCC p16$^{\text{INK4a}}$ antibody positive incidence, by gender.

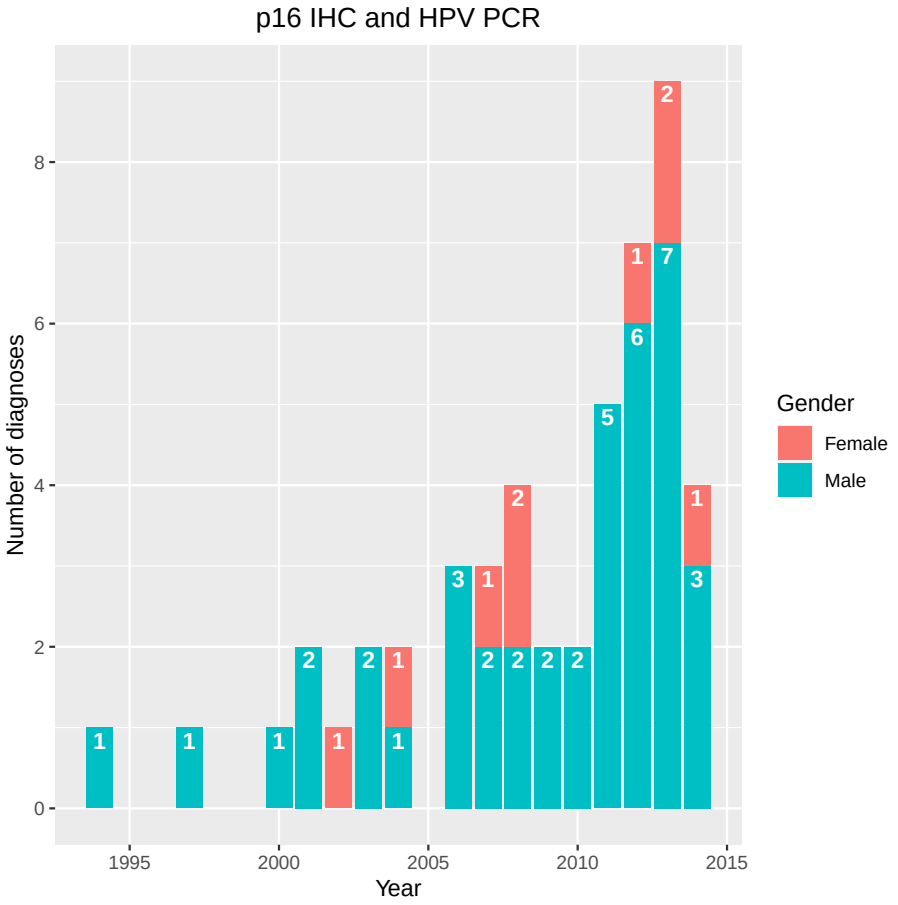


Figure 4: OPSCC simultaneously $p16^{INK4a}$ antibody and HPV DNA (as measured by PCR) positive incidence, by gender.

Patients are HPV positive iff both p16 antibodies are found with ICH and a significant number of HPV viral nuclear acids are found with PCR. The population residing in Iceland for half a year or longer grew during the study peroid on average by 1% a year.[Hagstofa, 2019] No statistics in this paper have been adjusted for population.

Overall incidence, estimated at 8 diagnoes/year at the end of the studied period grew by 3% to 10% per year (p-value 0.001). $p16^{INK4a}$ positive incidence, estimated at 7 diagnoes/year at the end of the studied period grew by 4% to 14% per year (p-value 0.0002). $p16^{INK4a}$ negative incidence is exceptionally stable. Simultaneously HPV PCR and p16 antibody positive incidence, estimated at 7 diagnoes/year at the end of the studied period grew by 6% to 19% per year (p-value 0.0002). In cases where either HPV PCR or $p16^{INK4a}$ is negative, incidence is exceptionally stable. Male incidence, estimated at 6 diagnoes/year

at the end of the studied period grew by 2% to 12% per year (p-value 0.003). Male p16 positive incidence, estimated at 5 diagnoes/year at the end of the studied period grew by 3% to 15% per year (p-value 0.003). Male HPV positive incidence, estimated at 6 diagnoes/year at the end of the studied period grew by 4% to 19% per year (p-value 0.003). Female incidence is exceptionally stable. Female p16 positive incidence is exceptionally stable. Female HPV positive incidence is exceptionally stable.

2 Survival

2.1 Overall survival

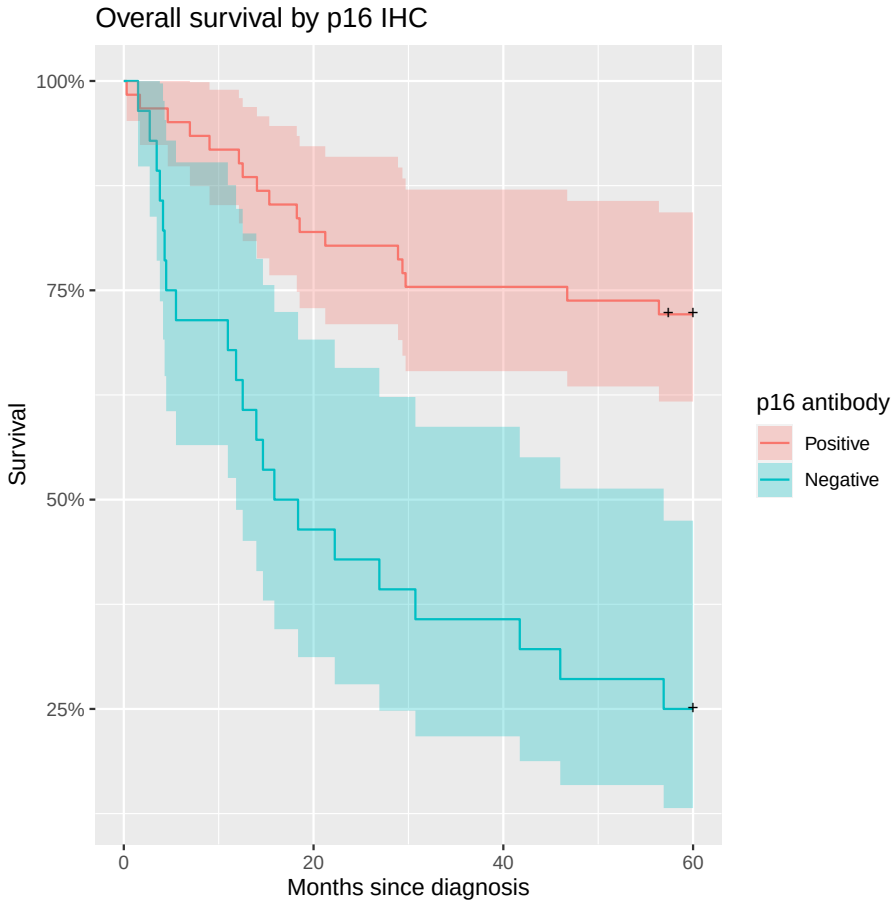


Figure 5: The colored shades cover the confidence region for the Kaplan-Meier survival curve of the same color. As expected, $p16^{INK4a}$ positive patients had better overall survival most of the time than did $p16^{INK4a}$ negative patients. A cross indicates a patient that was alive in August 2019 but was diagnosed in the preceding five years and had thus not yet reached the five year survival mark. Despite the survival curves crossing early on, Schoenfeld's partial residuals look and pass a chi-square test as independent of time, suggesting that the hazards of the two groups could be proportional. A Cox proportional hazards model indicates that $p16^{INK4a}$ positive patients are at only a third of the long-term death hazard of $p16^{INK4a}$ negative patients, and only at a fourth of the death hazard for the first five years after diagnosis (95% CI for the 5yr HR: 2–6×).

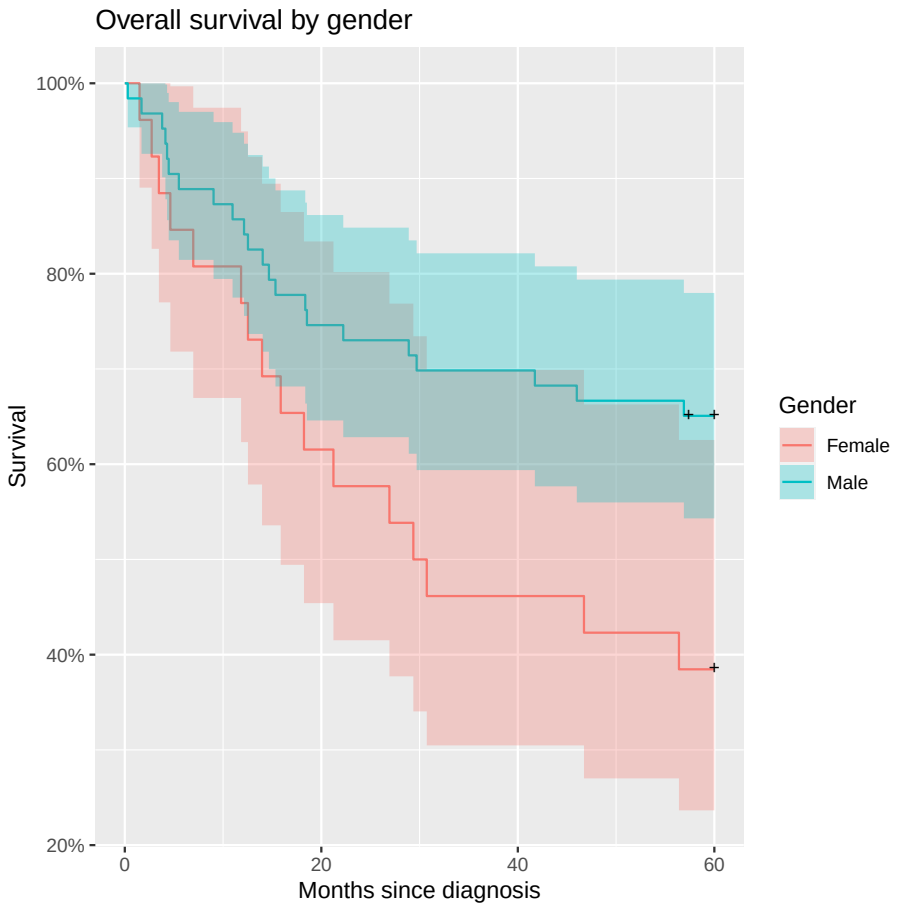


Figure 6: Five year survival by gender (Kaplan-Meier). If survival data had been right-censored two years after diagnosis, no statistically significant survival difference between the genders would have been found. Schoenfeld residues do, however, support the assumption of proportional hazard, and a Cox proportional hazards model fit to survival data up to August 2019 does find that females are after a diagnosis, with 95% "confidence," at somewhere between 8% and 300% greater death hazard than males.

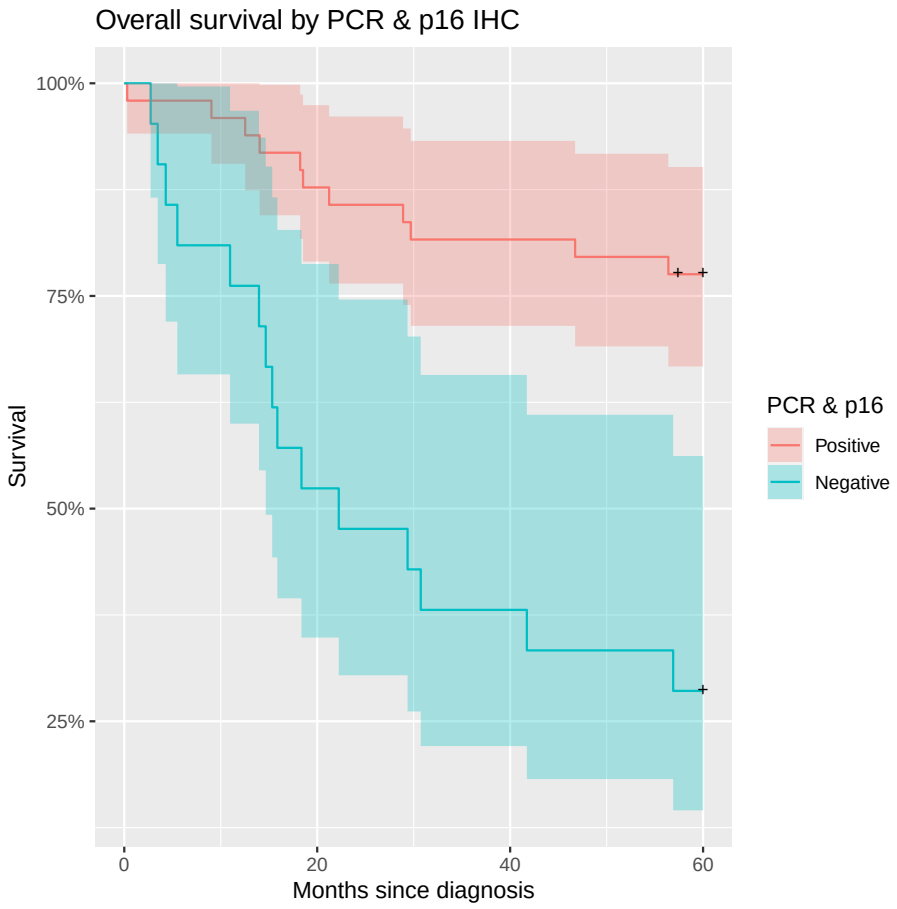


Figure 7: As expected, HPV positive patients had overall better survival than HPV negative patients.

Schoenfeld's partial residuals look and pass a chi-square test as independent of time, suggesting that the hazards of the two groups could be proportional. Despite the shortest surviving patient being $p16^{INK4a}$ positive, a Cox proportional hazards model indicates that $p16^{INK4a}$ positive patients are at only a fourth of the long-term death hazard of $p16^{INK4a}$ negative patients, and only at a fifth of the death hazard for the first five years after diagnosis (95% CI for the 5yr HR: 2–12×).

2.2 Disease-specific survival

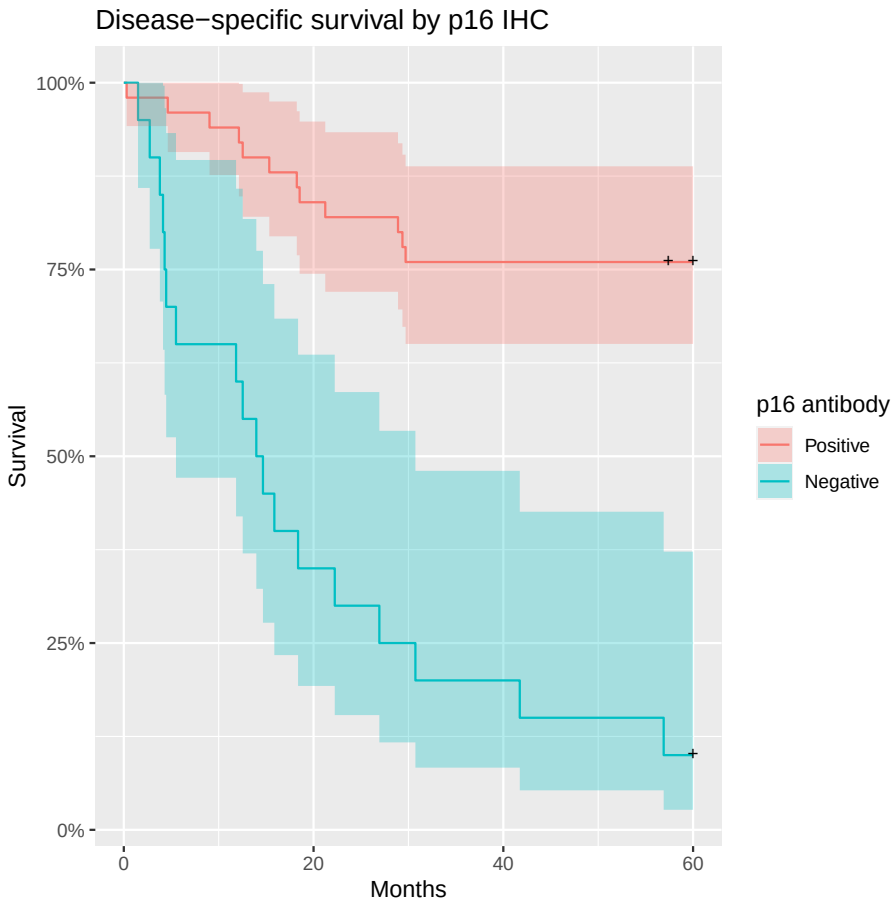


Figure 8: As expected, p16 positive patients had overall better disease-specific survival than p16 negative patients.

Disease-specific fatality hazard was four times higher for $p16^{INK4a}$ negative patients.

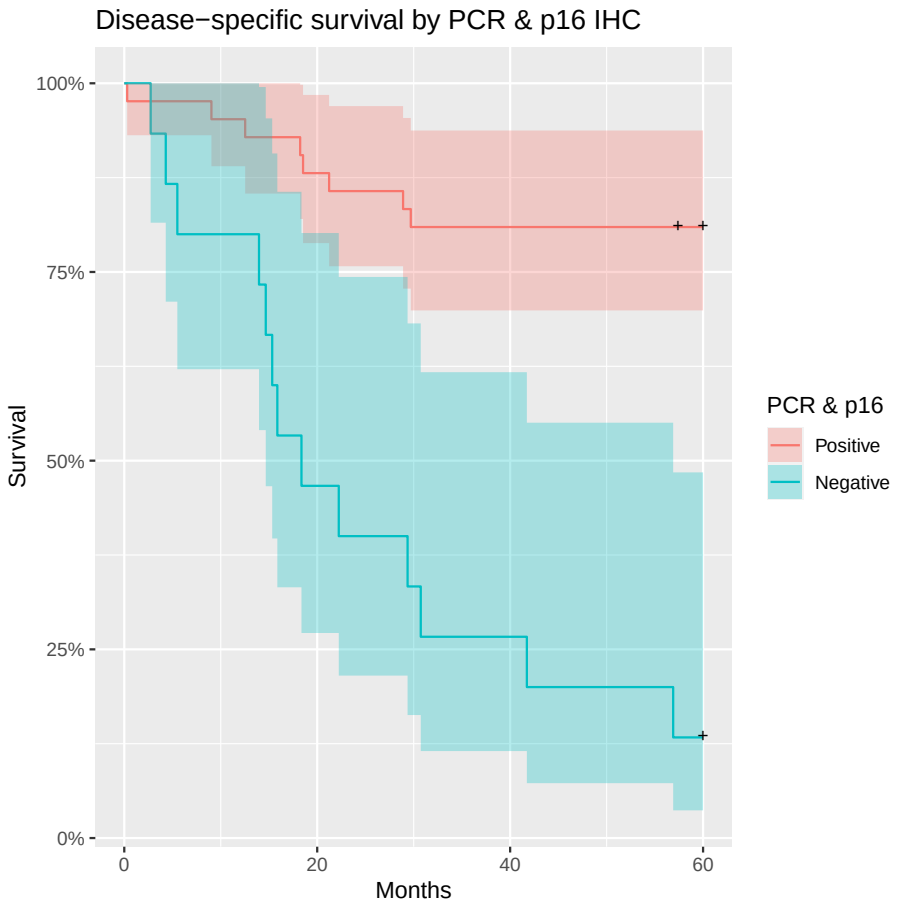


Figure 9: Disease-specific survival whether samples both produce a $p16^{INK4a}$ antibody stain and test HPV DNA positive using PCR.

As expected, HPV positive patients had overall better disease-specific survival than p16 negative patients.

3 Tumor location

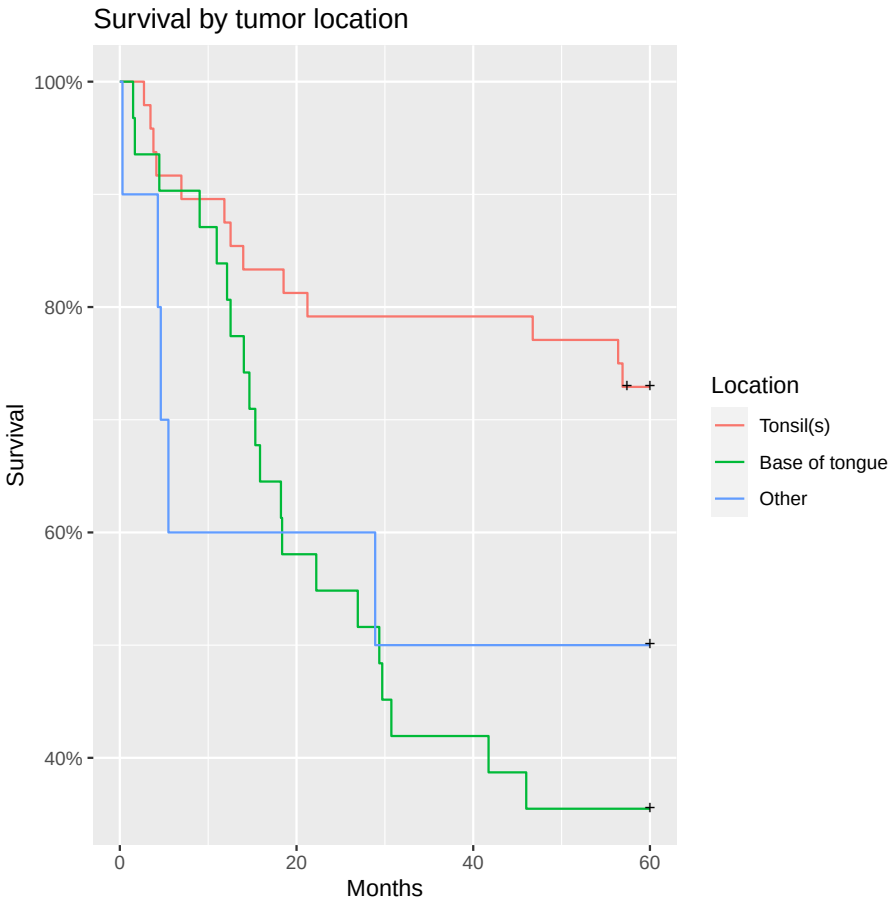


Figure 10: Kaplan-Meier survival graph for OPSCC patients by tumor location.

Patients were partitioned in three by (initial) tumor location: *tonsil*, *base of tongue* and *other*. Some patients in each group also had tumors elsewhere. A Cox proportional hazards model indicates that *tonsil* patients are at only half the death hazard of *base of tongue* patients (p-value if there was no difference: 0.02). The hazard ratio estimate would be the same even if survival data had been right-censored at two or five years after diagnosis.

	Female	Male
Tonsil(s)	13	35
Base of tongue	11	20
Other	2	8

	p16 -	p16 +
Tonsil(s)	9	39
Base of tongue	13	18
Other	6	4

Table 1: Tumor location by gender and $p16^{INK4a}$ stain.

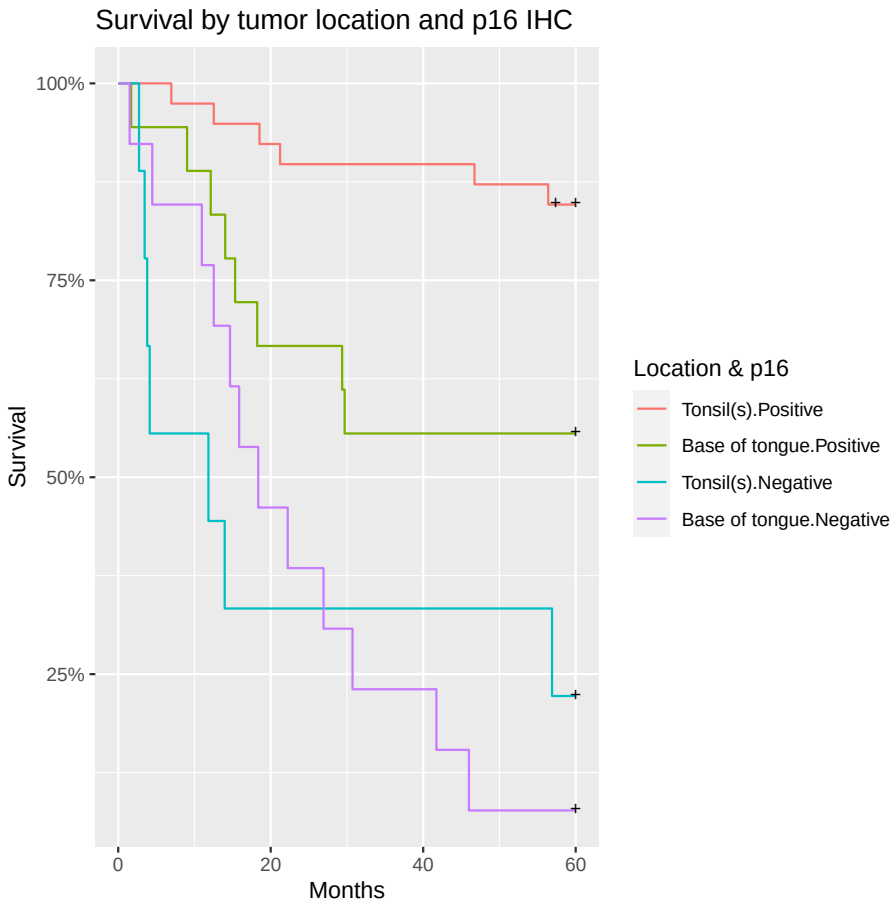


Figure 11: Kaplan-Meier survival graph for OPSCC patients by tumor location and $p16^{INK4a}$ immunohistochemistry result. The five year hazard from an oropharyngeal $p16^{INK4a}$ positive tumor is between a third and a sixteenth of the hazard from an oropharyngeal $p16^{INK4a}$ negative tumor, after correcting for more specific tumor location. In contrast, it's unclear if there is any survival difference between specific tumor location left after correcting for $p16^{INK4a}$ immunohistochemistry result. Right-censoring survivors two years after diagnosis yields a statistically significant difference, but the censoring five years after diagnosis does not. Schoenfeld residuals support the assumption of proportional hazards for up to five years, but no longer.

4 Correlation between smoking and HPV

	p16 +	p16 -	HPV +	HPV -
Smoking	39	23	34	17
Non-smoking	16	4	14	2

Table 2: Contingency table classifying patients by whether they have ever smoked and their p16 ICH result both alone and cross-checked against HPV PCR. There is not a discernible correlation between smoking and p16 ICH status (p-value 0.3).

	Tonsil(s)	Base of tongue	Other
Smoking	34	19	9
Non-smoking	13	7	0

Table 3: Contingency table classifying patients by whether they have ever smoked and their tumor location. There is not a discernible correlation between smoking and tumor location (p-value 0.2).

5 Tumor score and cancer stage

A notable change between the 7th and 8th editions of the TNM Cancer Staging Manual from the American Joint Committee on Cancer is that HPV status is factored into the cancer stage in the latter, but not the former. A tumor could be classified as stage IVa by the 7th edition and as stage I by the 8th edition.[1]

	N0	N1	N2a-c	N3	N3b
Positive	5	36	5	3	0
Negative	16	0	4	0	1

Table 4: Contingency table classifying patients by p16 stain and HPV PCR result and cancer N-score according to the American Joint Committee on Cancer 8th edition TNM Cancer Staging Manual. The N-score is partially defined in terms of HPV status.

	II	II(b)	III	IV	IV(a)	IV(b)
Positive	4	1	8	2	26	5
Negative	7	0	2	0	3	0

Table 5: Contingency table classifying patients by p16 stain and HPV PCR result and cancer stage according to the American Joint Committee on Cancer 7th edition TNM Cancer Staging Manual. The cancer stage is not defined in terms of HPV status. There is a correlation between HPV and cancer stage (p-value 0.006).

[1]Hoffmann M, Tribius S. HPV and Oropharyngeal Cancer in the Eighth Edition of the TNM Classification: Pitfalls in Practice. *Transl Oncol.* 2019;12(8):1108-1112. doi:10.1016/j.tranon.2019.05.009

	(unknown)	I	II	III	IV	IV(a)	IV(b)
Positive	0	33	10	5	1	0	0
Negative	1	3	8	3	0	5	1

Table 6: Contingency table classifying patients by p16 stain and HPV PCR result and cancer stage according to the American Joint Committee on Cancer 8th edition TNM Cancer Staging Manual. The cancer stage is partially defined in terms of p16 stain result. There is a correlation between HPV and cancer stage (p-value 0.0002).

	HPV +	HPV -	I	II	III	IV	IV(a)	IV(b)
Female	15	11	11	8	5	0	1	0
Male	46	17	30	14	10	1	6	2

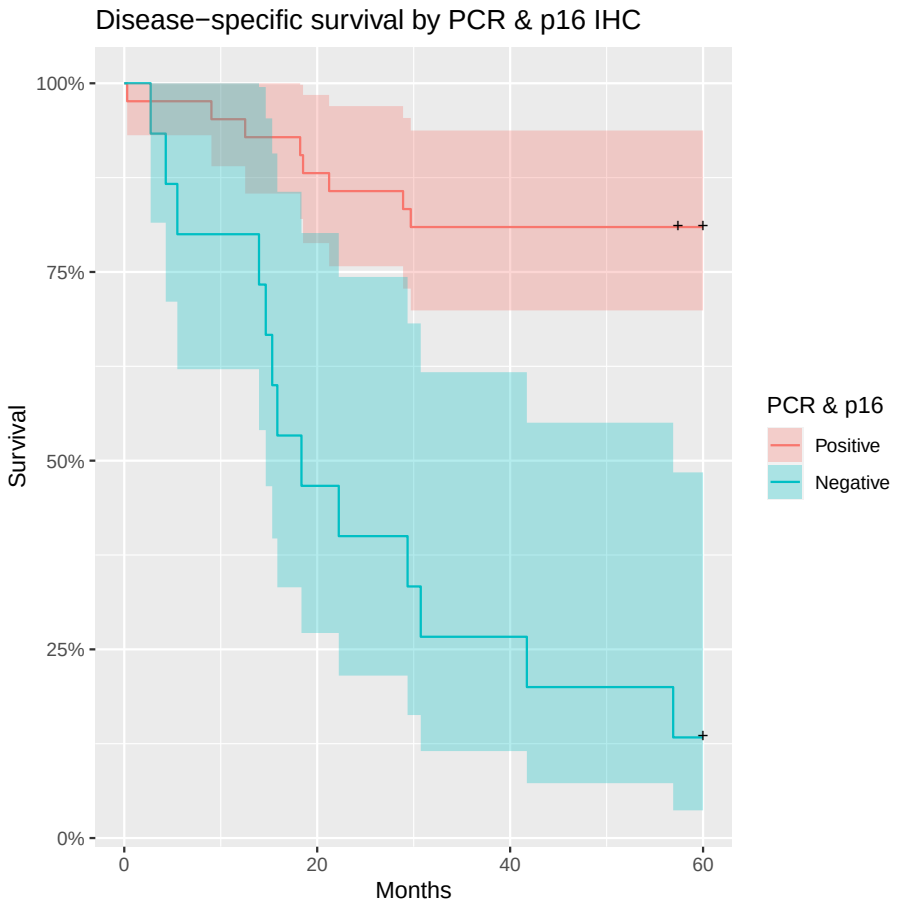
Table 7: Combined $p16^{INK4a}$ antibody stain and HPV DNA PCR result and TNM cancer stage by gender.

6 Gender

	Diagnoses	Male	Female
1994 - 2004	28	20	8
2004 - 2014	61	43	18

7 A note on p-values

All hypothesis tests were performed at significance level $\alpha = 5\%$. The null hypotheses rejected with p-values close to 0.05 are rejected because they are opposed by biological arguments and clinical experience. The p-values in this paper are heavily skewed towards zero, with only one p-value over half. An Anderson-Darling shows that the p-values that are presumed to be independent do not come from the uniform distribution (p-value 0.04, would decrease below 0.00005 if interdependent p-values were included).



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