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Original Article

A Prospective Cohort Study of Human Papillomavirus-Driven Oropharyngeal Cancers: Implications for Prognosis and Immunisation

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Abstract

Aims: Oropharyngeal cancer (OPC) is increasing on a global scale, including the component driven by high-risk human papillomavirus (HR-HPV); contemporary data that provides insight into the prognosis of this disease in addition to the fraction attributable to HR-HPV are essential to inform primary and secondary disease management strategies.
Materials and methods: A population-based cohort of 235 patients diagnosed with OPC between 2013 and 2015 in Scotland was assessed for HPV status using molecular genotyping. Associations between HR-HPV status and key clinical and demographic variables were estimated using the Pearson chi-squared test. Rates of overall survival and progression-free survival were estimated and visualised using Kaplan–Meier curves.
Results: HPV DNA (largely HPV 16) was identified in 60% of cases. After adjustment for age, gender, deprivation, smoking, alcohol consumption and tumour stage, patients with HR-HPV-positive OPC had an 89% reduction in the risk of death (hazard ratio = 0.11, 95% confidence interval 0.05–0.25) and an 85% reduction in the risk of disease progression (hazard ratio = 0.15, 95% confidence interval 0.07–0.30). HPV positivity was not associated with age, deprivation or smoking status, whereas those who reported excess alcohol consumption were less likely to be positive for HR-HPV.
Conclusions: The prevalence of HR-HPV-associated OPC is high in Scotland and strongly associated with dramatically improved clinical outcomes, including survival. Demographic/behavioural variables did not reliably predict HPV positivity in this cohort, which underlines the importance of laboratory confirmation. Finally, the dominance of HPV 16 in OPC indicates the significant impact of prophylactic immunisation on this disease.

Key words: Cancer; HPV; oropharyngeal; prevalence; prognosis

Introduction

Oropharyngeal cancer (OPC) is increasing globally and the rate has doubled in the past 15 years in the UK, with clinicians describing it as an 'epidemic' [1–3]. In Scotland, OPC is one of the most increasingly incident cancers, especially among men [4]. Tobacco use and alcohol consumption remain the major risk factors for head and neck cancers. However, an increase in high-risk human papillomavirus (HR-HPV)-driven OPC has also been observed [5,6]. Analyses of Scottish Cancer Registry head and neck cancer incidence trends over the past 40 years show that the rate of laryngeal cancer, which is strongly associated with tobacco consumption, has remained essentially stable. Oral cancer, which is associated with alcohol consumption, is steadily increasing, whereas OPC has risen comparatively more

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rapidly, with a rate that has increased nearly three-fold in recent years and is projected to rise further [7]. In relation to risk factors in head and neck cancer in Scotland, these trends indicate a continued role of tobacco, an increasing role of alcohol and a dramatic and relatively new role of HPV [8].

The fraction of OPC associated with HPV varies significantly with geographic location; in the USA prevalence is around 60%, whereas in South America the attributable fraction is less than 5% [9]. A study of about 1500 archived OPC cases from 11 participating centres in the UK [10] (diagnosed between 2002 and 2011) showed that about 50% were HR-HPV DNA-positive, largely for HPV type 16. Furthermore, the main HPV genotype identified in oral rinse samples from the general Scottish population was HPV 16 [11]. HPV is thought to be acquired through sexual exposure [12,13], although the natural history of HPV-associated OPC is not as well defined as other HPV-associated neoplasms, such as cervix. Although the increase in OPC incidence is of clear concern, patients with HPV-driven OPC generally have a better prognosis compared with those patients who are HPV-negative [14]. Of note, the International Collaboration on Oropharyngeal Cancer Network for Staging (ICON–S) has developed a new staging classification system informed by HPV status that recognises the improved clinical outcomes and this has now been incorporated into the eighth edition of the TNM classification [14].

Studies of North American populations suggest that the sociodemographic status of head and neck cancer patients may also be changing, with an increasing proportion of patients with OPC diagnosis being younger and more socioeconomically affluent [15]. This phenomenon is not mirrored in Scotland, as recent national cancer registry data show that patients from the most deprived areas consistently had the highest rates of OPC [8]. While the HPV status of OPCs is not available in Scottish Cancer Registry data, the association between deprivation and cancer rates may reflect the overwhelming influence of tobacco and alcohol consumption in head and neck cancer oncogenesis in Scotland. The Scottish population has historically had a high rate of smoking, with 45% of adults reporting as current or ex-regular cigarette smokers (as of 2016), with about 60% residing in the most deprived areas [16]. Excessive alcohol consumption in Scotland is also high, with one in four adults consuming harmful levels in 2016 [16].

Prior to 2013, HPV testing of OPC in Scotland was carried out opportunistically at the treating clinician's request. Thereafter it was offered as a service to all Scottish health boards via the National Reference Laboratory facility. This evaluation is one of the first to assess outcomes in a prospective, population-based cohort of OPC patients, where HPV genotyping has been performed by a standardised methodology in a centralised location. We present data on the prevalence of HPV in OPC in Scotland and the association with key social variables and clinical indicators/outcomes. Furthermore, we investigate whether improved outcome associated with HPV-driven OPC is reflected in populations where rates of tobacco use and alcohol are particularly high, such as the West of Scotland.

Materials and Methods

Dimension and Characteristics of the Patient Cohort

All OPCs, including subsites of the tonsil, base of tongue, soft palate and pharyngeal walls, diagnosed in the West of Scotland Cancer Network between April 2013 and June 2015 underwent pathology review and prospective HPV genotyping as part of routine clinical care. The health boards included in the present analysis covered the following locations: West Dunbartonshire, East Dunbartonshire, East Renfrewshire, Glasgow City, Inverclyde, Renfrewshire, Forth Valley, Lanarkshire, Ayrshire and Arran, which cover a population of about 2.5 million people. All individuals accessing health care in Scotland are assigned a unique 10-digit number, which allows linkage of clinical, social and laboratory data. Sociodemographic and clinical data were extracted from the West of Scotland Cancer Network Head and Neck Cancer Quality Performance Indicators, which are collected by all health boards in Scotland and used to drive quality improvement in cancer care across NHS Scotland and clinical records. The variables collected were: date of diagnosis (taken as the date of the initial diagnostic biopsy sample collection), age at diagnosis, cancer stage, seventh edition TNM classification, eighth edition TNM classification for HPV-positive OPCs/ICON–S [14], which takes into account HPV status, date of relapse if occurred and, if applicable, date and cause of death, smoking status (never versus ever) at the time of diagnosis and self-reported alcohol excess. Alcohol excess is defined by NHS Scotland as consuming alcohol at $\geq$21 units or more per week for men and $\geq$14 units per week for women or documentation of 'heavy', 'excessive' or 'dependency' on alcohol within clinical records. In addition, area-based socioeconomic status was obtained, via the Scottish Index of Multiple Deprivation (SIMD), where 1 and 5 are the most and least deprived, respectively. Treatment intention and modality were based on clinician preference and patient factors, including age, frailty and wishes. Data were extracted by treating clinicians and all patient identifiable data were removed before statistical analysis. Study governance and ethical considerations were through NHS Greater Glasgow and Clyde Research and Development Office, the Clinical Effectiveness Team and a data sharing agreement with the West of Scotland Cancer Network. Data were censored at November 2016.

Nucleic Acid Extraction and Human Papillomavirus Genotyping

A formalin-fixed, paraffin-embedded block was selected and a 10 μm section obtained for nucleic acid extraction. Nucleic acid extraction was carried out using reagents within the DNA mini kit (Qiagen, Hilde, Germany) with an adaptation to the protocol to maximise recovery of HPV DNA [17]. Subsequently, HPV genotyping was carried out using polymerase chain reaction (PCR) and the Luminex-based Optiplex HPV genotyping test (Diamex, Heidelberg, Germany). This assay detects 24 HPV types, including all

established HR types and, as a check for specimen adequacy, incorporates a cellular housekeeping control (betaglobin). This assay was used for the recent UK prevalence study of 1500 OPC referred to earlier [10]. Agreement of the assay with p16INK4a immunohistochemistry has been shown to be 90% in oropharyngeal samples with no significant difference in the distribution of discordant results between the two tests. Furthermore, positivity according to this assay has been shown to correlate with cause-specific survival in Scottish cohorts [10,18]. All testing was carried out at a centralised reference laboratory.

Analysis of Human Papillomavirus Status with Clinical Outcomes

Associations between HR-HPV status and clinical and demographic variables were estimated using the Pearson chi-squared test. Both unadjusted and adjusted odds ratios for HPV positivity and 95% confidence intervals were calculated using logistic regression. A linear trend test was carried out to investigate whether there was an increasing trend in the risk of HPV-positive OPC with increasing age. Rates of overall survival and progression-free survival (PFS) were determined and univariate comparisons visualised using Kaplan–Meier curves. The univariate impact of each variable on survival was measured using Cox proportional hazards regression. An adjusted model was created to obtain the adjusted hazard ratios for HR-HPV status with adjustment of the following covariates: gender, age at diagnosis (<50, 50–59, 60–69, 70–79 and 80+), SIMD, smoking and alcohol status and TNM classification 7 of cancer. Subset analysis on the HPV-positive patients was carried out to obtain univariate and adjusted hazard ratios for TNM classification 8/ICON–S stage.

All statistical analyses were carried out using R version 3.2.3. All variables with P values < 0.05 were considered to be statistically significant.

Results

Description of Cohort

In total, 235 patients were diagnosed with OPC between April 2013 and June 2015. Technically valid HPV results were obtained for 229 patients, with six excluded due to non-amplification of the cellular housekeeping gene. Only 22% of the 229 OPC patients were women and the median age was 60 years (interquartile range 54–69). Most of the cohort was resident in a deprived area (37.6% in SIMD1 and 21% in SIMD2). Most reported having ever smoked tobacco (65%), with 31% reporting drinking alcohol to excess (Table 1).

Human Papillomavirus Positivity and Association with Extrinsic Risk Factors

HPV DNA was identified in 60% of the cases (136/229). Single infections with HPV 16, 18, 33, 35 were identified in 127, two, one and one case, respectively. The occurrence of multiple infections of 16/6, 16/59, 18/82, 6/16/33 and 18/59/82 all occurred in a single case each. All bar one of the HPV-positive cases included at least one established HR-HPV type.

The influence of gender and risk factors are summarised in Table 1. Men were twice as likely as women to have an HPV-positive tumour (odds ratio 2.44, 95% confidence interval 1.18–5.05, $P = 0.02$). Those who reported drinking alcohol to excess were 70% less likely to be HPV-positive (odds ratio 0.30, 95% confidence interval 0.14–0.63, $P = 0.001$). HPV positivity was not associated with social deprivation as measured by SIMD ($P = 0.2$) or age (linear trend $P = 0.7$) or smoking status ($P = 0.1$). The interaction between smoking and alcohol was not significant in univariate regression for the association with HR-HPV ($P = 0.99$).

Human Papillomavirus Status and Survival

In total, 35 patients were excluded from the survival analysis: one was lost to follow-up and 34 received treatment intent of best support palliative care due to frailty and were unable to tolerate treatment. Post-exclusions, the median follow-up time of the remaining cohort after OPC diagnosis was 2.2 years (range from 19 days to 3.4 years). In total, 23% of patients (45/194) died and 28% of patients (55/194) had disease progression during the follow-up period. The 1-year overall survival rate for HPV-negative patients was 75.7%, whereas for HPV-positive patients overall survival was 95.2%. The 1-year PFS rate for HPV-negative and HPV-positive patients was 67.1% and 92.7%, respectively (Figures 1 and 2). One-year overall survival rates for the patients who smoked was 86.4% compared with 90.2% for the patients who reported never smoking. The 1-year overall survival rate for the patients who drank alcohol to excess was 80.4% compared with 91.5% for those who did not. In our data we observed an increase in the point estimates for death and disease-free survival as TNM classification 7 increased from stage I to IV, but use of ICON–S stage for the HPV-positive population was not discriminatory.

Univariate analysis also showed that HPV-negative status and alcohol consumption were significantly associated with increased risk of death (both $P < 0.001$; Table 2). After adjustment for age, gender, SIMD, smoking and drinking status and tumour stage, patients with HR-HPV-positive OPC had an 89% reduction in the risk of death (hazard ratio = 0.11, 95% confidence interval 0.05–0.24, $P < 0.001$; Table 2), and had an 84% reduction in the risk of disease progression (hazard ratio = 0.15, 95% confidence interval 0.07–0.30, $P < 0.001$; Table 3) compared with those with HPV-negative OPC. The risk of death increased with age in the fully adjusted model. Patients who reported drinking alcohol to excess had 2.3 times the risk of death (hazard ratio overall survival = 2.33, 95% confidence interval 1.06–5.18, $P = 0.04$) compared with those who did not drink alcohol to excess.

Discussion

We conducted a detailed population-based evaluation to examine the impact of demographic, behavioural and viral

Table 1
Factors associated with high-risk human papillomavirus (HR-HPV) among oropharyngeal cancer (OPC) cases

Variable	Level	Number ($n = 229$)*		HR-HPV+ ($n = 136$)		Unadjusted				Adjusted (full model)†			
		n	%	*n*	%	OR	95% CI lower	95% CI upper	*P* values	OR	95% CI lower	95% CI upper	*P* values
Gender	Female	51	22.3	25	49.0	1				1			
	Male	178	77.7	111	62.4	1.72	0.92	3.23	0.09	2.44	1.18	5.05	0.02
Age	<50	27	11.8	20	74.1	1							
	50–59	86	37.6	58	67.4	0.73	0.27	1.92	0.5				
	60–69	60	26.2	30	50.0	0.35	0.13	0.95	0.04				
	70–79	49	21.4	25	51.0	0.36	0.13	1.02	0.05				
	80 and over	7	3.1	3	42.9	0.26	0.05	1.48	0.1				
	Age in years (19–91)	229				0.97	0.95	1	0.04 (trend)	0.99	0.97	1.02	0.7 (trend)
SIMD	1: most deprived	86	37.6	44	51.2	1				1			
	2	48	21.0	27	56.3	1.23	0.6	2.5	0.6	1.01	0.44	2.33	1.0
	3	35	15.3	26	74.3	2.76	1.16	6.57	0.02	4.24	1.45	12.35	0.01
	4	29	12.7	20	69.0	2.12	0.87	5.18	0.1	1.75	0.64	4.75	0.3
	5: least deprived	19	8.3	13	68.4	2.07	0.72	5.94	0.2	1.39	0.4	4.86	0.6
	Missing	12	5.2	6	50.0					0.94	0.18	5	0.9
Smoking status	No	63	27.5	50	79.4	1				1			
	Yes	149	65.1	77	51.7	0.28	0.14	0.55	0.0003	0.52	0.23	1.17	0.1
	Missing	17	7.4	9	52.9					1.12	0.18	6.94	0.9
Alcohol status	No	129	56.3	91	70.5	1				1			
	Yes	71	31.0	30	42.3	0.31	0.17	0.56	0.0001	0.3	0.14	0.63	0.001
	Missing	29	12.7	15	51.7					0.45	0.12	1.69	0.2
Smoking and alcohol	No/either	138	60.3	98	72.1	1							
	Both	66	28.8	27	19.8	0.28	0.15	0.52	0.00005				
	Missing	25	10.9	11	8.1								
TNM stage	I	13	5.7	2	15.4	1				1			
	II	26	11.4	11	42.3	4.03	0.74	21.98	0.1	10.94	1.6	74.91	0.01
	III	20	8.7	13	65.0	10.21	1.75	59.65	0.01	23.37	3.09	176.86	0.002
	IVa	148	64.6	100	67.6	11.46	2.44	53.74	0.002	23.8	3.99	141.98	0.001
	IVb	12	5.2	6	50.0	5.5	0.84	36.2	0.08	17.65	1.94	160.87	0.01
	IVc	4	1.7	2	50.0	5.5	0.46	65.16	0.2	8.38	0.63	112.14	0.1
	Missing	6	2.6	2	33.3					5.81	0.39	86.24	0.2

CI, confidence interval; OR, odds ratio; SIMD, Scottish Index of Multiple Deprivation.

* Valid HPV results available for 229/235 cases.

† Adjusted for all variables in the table except for treatment. It is highly associated with TNM stage and therefore not included in the adjusted model to avoid collinearity.

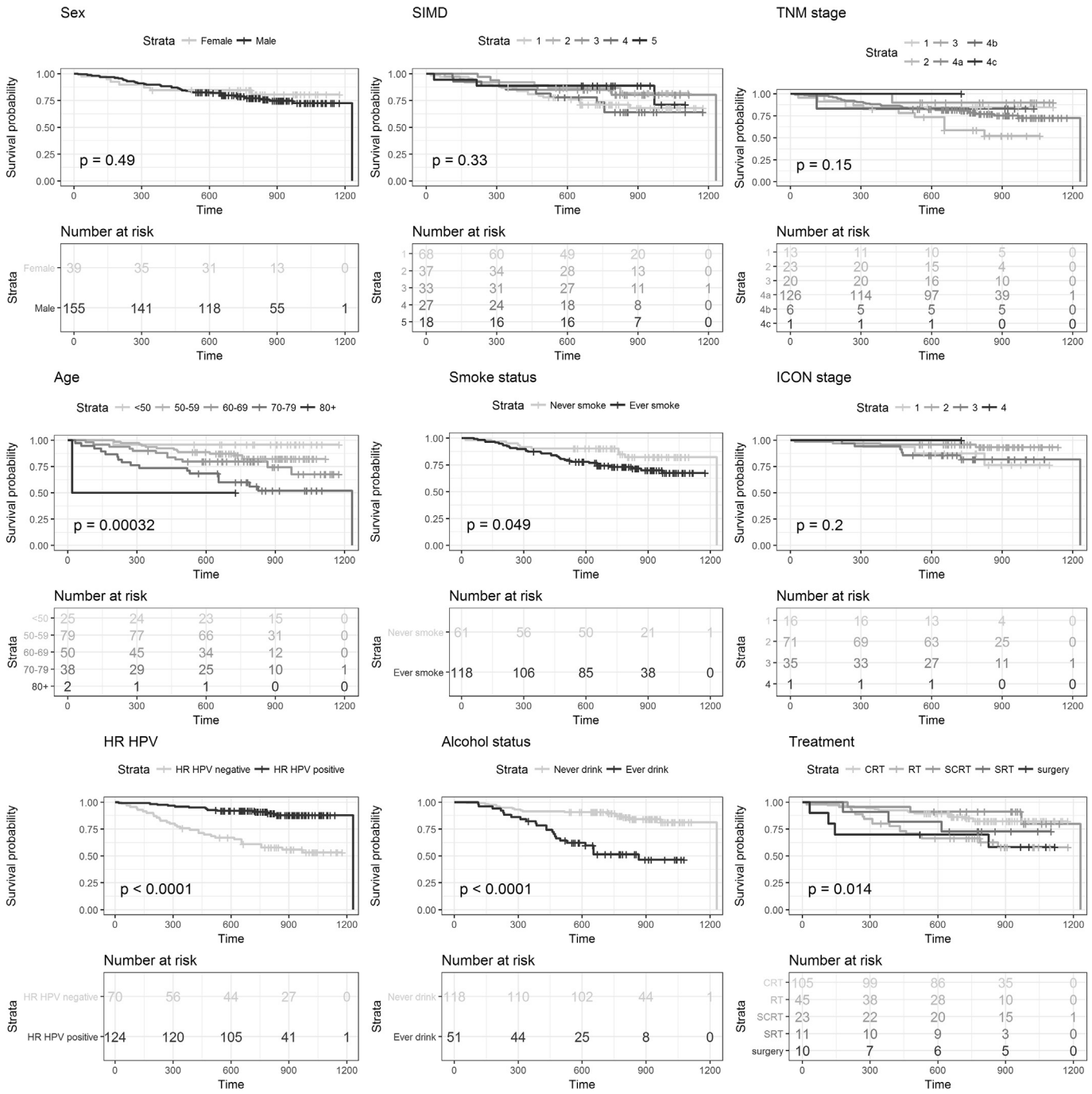

Fig 1. Survival plots for overall survival (plot for International Collaboration on Oropharyngeal Cancer Network for Staging [ICON–S] stage are only for the subset of high-risk human papillomavirus-positive patients).

factors associated with OPC in a context where both disease prevalence and non-viral risk factors (alcohol consumption and smoking) are high. We have shown that HR-HPV prevalence among OPCs in the West of Scotland was 60% and that HR-HPV positivity was higher in men compared with women and lower among those who reported drinking alcohol to excess. We also confirmed that in our population HR-HPV status was strongly associated with improved overall survival and PFS. It was of interest that individuals reporting tobacco exposure were as likely to have cancers associated with HR-HPV as those individuals who reported no history of tobacco use, a finding at variance with other previously reported UK data [19].

Studies suggest that the incidence of HR-HPV detection in OPCs varies widely depending on geographical location and the period of time. We report HR-HPV rates of 49%

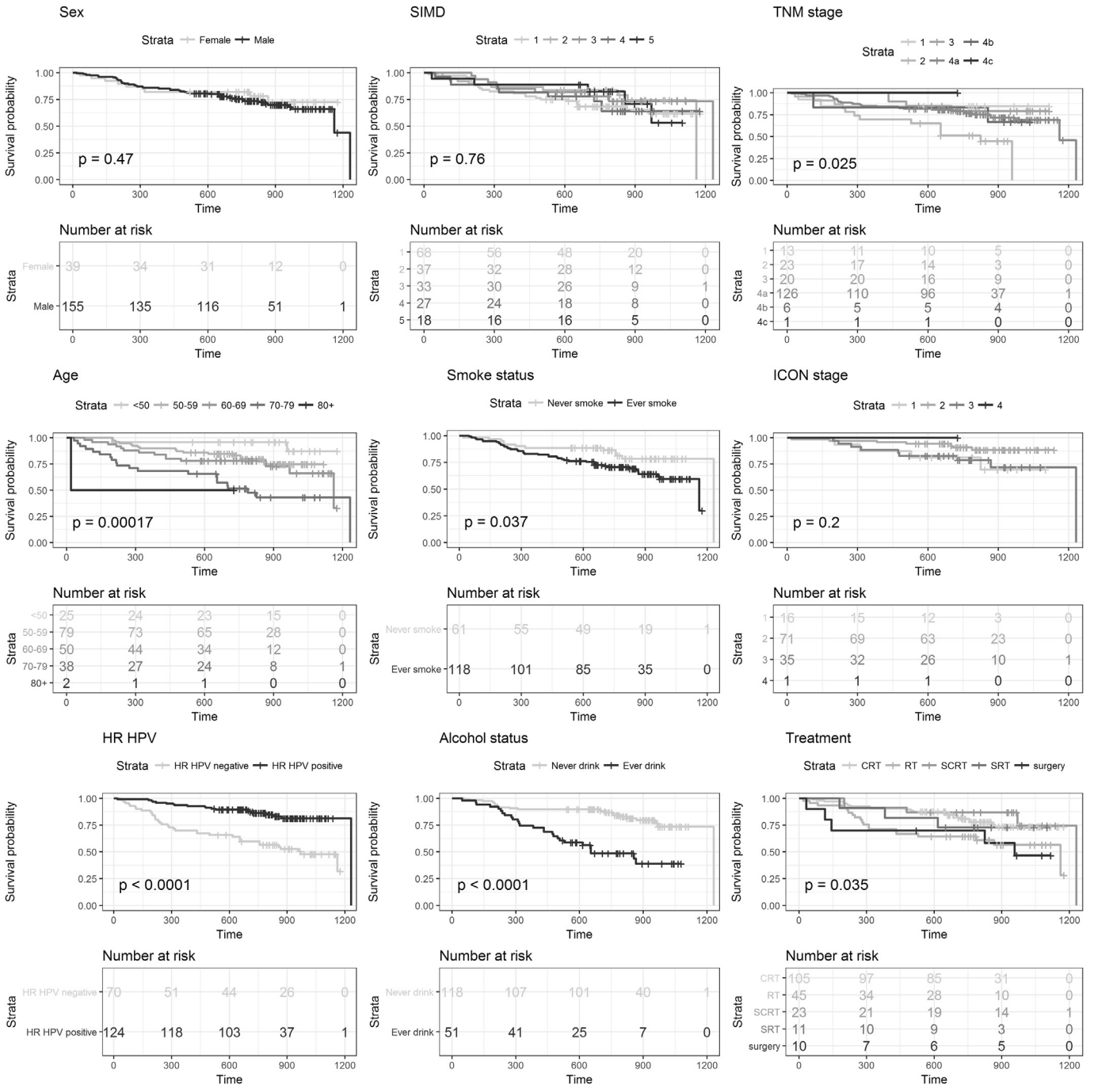

Fig 2. Survival plots for progression-free survival (plot for International Collaboration on Oropharyngeal Cancer Network for Staging [ICON–S] stage are only for the subset of high-risk human papillomavirus-positive patients).

among women and 62.4% among men, with an overall positivity of 60%. These are broadly in line with the UK-based results of Schache *et al.* [10] and reflect a more recent period of assessment. Beyond the UK, reports of HR-HPV prevalence in OPCs in the most recent decade range from 6.1% in Spain [20], 38% in the Netherlands [21] and 62% in Denmark [22]. US estimates are consistently higher at around 60–70% [9]. A confounder to cross-study comparison is the different methods by which HPV-positive status is defined, which vary across studies and include nucleic acid amplification tests, immunohistochemistry with p16INK4a, *in situ* hybridisation and combinations thereof. In this work we used a sensitive PCR-based assay to confirm HPV status rather than p16INK4a; this assay has been shown to have a high level of agreement (90%) with p16INK4a immunohistochemistry, with no difference in the

Table 2
Hazard ratios (HR) for overall survival

Variable	Level	Number ($n = 194$)*	Number with all-cause death	Person years of follow-up	Rate per 100 person years	95% CI lower	95% CI upper	Univariate HR	95% CI lower	95% CI upper	*P* value	Adjusted (full) HR†	95% CI lower	95% CI upper	*P* value
HR-HPV status	Negative	70	31	132.7	23.4	15.9	33.1	1.00				1.00			
	Positive	124	14	269.8	5.2	2.8	8.7	0.20	0.11	0.39	<0.00001	0.11	0.05	0.24	<0.00001
Gender	Female	39	7	80.8	8.7	3.5	17.9	1.00				1.00			
	Male	155	38	321.8	11.8	8.4	16.2	1.33	0.59	2.99	0.5	1.04	0.44	2.69	0.9
Age	<50	25	1	60.3	1.7	0.04	9.2	1.00				1.00			
	50–59	79	13	172.2	7.5	4.0	12.9	4.45	0.58	34.04	0.2	1.55	0.34	14.88	0.6
	60–69	50	12	98.5	12.2	6.3	21.3	7.25	0.94	55.82	0.06	1.92	0.40	18.93	0.5
	70–79	38	18	69.6	25.9	15.3	40.9	14.59	1.94	109.70	0.01	10.39	2.42	97.07	0.0007
	80 and over	2	1	2.0	48.9	1.2	272.6	28.87	1.80	463.90	0.02	104.44	6.53	1744.56	0.003
SIMD	1: most deprived	68	20	132.0	15.1	9.3	23.4	1.00				1.00			
	2	37	6	78.4	7.7	2.8	16.7	0.51	0.20	1.26	0.1	0.66	0.21	1.81	0.4
	3	33	7	70.0	10.0	4.0	20.6	0.57	0.23	1.42	0.2	1.30	0.47	3.25	0.6
	4	27	9	53.1	17.0	7.8	32.2	1.11	0.51	2.45	0.8	2.77	1.09	6.70	0.03
	5: least deprived	18	3	38.6	7.8	1.6	22.7	0.51	0.15	1.73	0.3	0.91	0.23	2.70	0.9
	Missing	11	0	30.4	0.0	0.0	12.1					0.11	0.00	1.18	0.07
Smoking status	No	61	10	129.0	7.8	3.7	14.3	1.00				1.00			
	Yes	118	34	235.7	14.4	10.0	20.2	2.06	0.99	4.30	0.05	1.64	0.64	4.45	0.3
	Missing	15	1	37.9	2.6	0.1	14.7					4.23	0.28	48.34	0.3
Alcohol status	No	118	18	256.9	7.0	4.2	11.1	1.00				1.00			
	Yes	51	24	84.7	28.3	18.1	42.1	4.34	2.32	8.12	<0.00001	2.32	1.06	5.18	0.04
	Missing	25	3	61.0	4.9	1.0	14.4					0.69	0.12	2.82	0.6
TNM stage	I	13	2	27.6	7.2	0.9	26.2	1.00				1.00			
	II	23	10	41.2	24.3	11.6	44.7	3.20	0.70	14.64	0.1	3.20	0.86	17.30	0.08
	III	20	2	45.5	4.4	0.5	15.9	0.60	0.09	4.29	0.6	3.85	0.53	28.22	0.2
	IVa–c‡	133	31	273.5	11.3	7.7	16.1	1.50	0.36	6.26	0.6	9.97	2.57	56.41	0.0005
	Missing	5	0	14.8	0.0	0.0	24.9								
ICON–S stage‖	I	16	3	34.3	8.7	1.8	25.6	1.00							
	II	71	4	156.8	2.6	0.7	6.5	0.30	0.07	1.34	0.1				
	III–IV§	36	7	75.7	9.2	3.7	19.0	0.97	0.24	3.87	1.0				
	Missing	1	0	3.0	0.0	0.0	122.9								

CI, confidence interval; HR-HPV, high-risk human papillomavirus; ICON–S, International Collaboration on Oropharyngeal Cancer Network for Staging; SIMD, Scottish Index of Multiple Deprivation.

* Individual removed from overall survival analysis as lost to follow-up (no date of death and no date alive); individuals also removed from overall survival analysis as treatment was best-supportive care.

† Adjusted for all variables in the table except for ICON stage. They are highly associated with TNM stage and are therefore not included in the adjusted model to avoid collinearity.

‡ For TNM stage, IVa–c combines the levels IVa (126 cases, 30 died), IVb (6 cases, 1 died) and IVc (1 case, 0 died).

§ For ICON stage, III–IV combines the levels III (35 cases, 7 died) and IV (1 case, 0 died).

‖ Only select HR-HPV-positive cases, $n = 124$.

Table 3
Hazard ratios (HR) for progression-free survival

Variable	Level	Number ($n = 194$)	Number recur or die	Person years of follow-up	Rate per 100 person years	95% CI lower	95% CI upper	Univariate HR	95% CI lower	95% CI upper	*P* value	Adjusted (full) HR*	95% CI lower	95% CI upper	*P* value
HR-HPV status	Negative	70	35	129.6	27.0	18.8	37.6	1.00				1.00			
	Positive	124	20	263.2	7.6	4.6	11.7	0.27	0.16	0.48	<0.00001	0.15	0.07	0.30	<0.00001
Gender	Female	39	9	79.8	11.3	5.2	21.4	1.00				1.00			
	Male	155	46	313.0	14.7	10.8	19.6	1.30	0.64	2.66	0.5	0.98	0.47	2.24	1.0
Age	<50	25	2	60.1	3.3	0.4	12.0	1.00				1.00			
	50–59	79	17	168.1	10.1	5.9	16.2	3.12	0.72	13.54	0.1	1.46	0.41	7.79	0.6
	60–69	50	14	97.9	14.3	7.8	24.0	4.37	0.99	19.25	0.051	1.59	0.42	8.71	0.5
	70–79	38	21	64.6	32.5	20.1	49.7	9.55	2.23	40.91	0.002	8.65	2.57	44.83	0.0002
	80 and over	2	1	2.0	48.9	1.2	272.6	15.93	1.43	176.98	0.02	45.72	3.49	454.44	0.007
SIMD	1: most deprived	68	23	129.0	17.8	11.3	26.8	1.00				1.00			
	2	37	9	76.7	11.7	5.4	22.3	0.65	0.30	1.41	0.3	1.06	0.42	2.49	0.9
	3	33	9	67.1	13.4	6.1	25.5	0.67	0.30	1.49	0.3	1.35	0.55	3.05	0.5
	4	27	9	52.1	17.3	7.9	32.8	0.95	0.44	2.05	0.9	2.10	0.85	4.88	0.1
	5: least deprived	18	5	37.5	13.3	4.3	31.1	0.76	0.29	2.00	0.6	1.34	0.45	3.39	0.6
	Missing	11	0	30.4	0.0	0.0	12.1					0.05	0.00	0.49	0.01
Smoking status	No	61	12	125.4	9.6	4.9	16.7	1.00				1.00			
	Yes	118	41	231.3	17.7	12.7	24.0	2.00	1.03	3.90	0.04	1.70	0.73	4.12	0.2
	Missing	15	2	36.0	5.5	0.7	20.0					11.43	1.30	95.61	0.0003
Alcohol status	No	118	23	250.9	9.2	5.8	13.8	1.00				1.00			
	Yes	51	27	82.3	32.8	21.6	47.7	3.84	2.17	6.79	<0.00001	2.08	0.99	4.33	0.1
	Missing	25	5	59.5	8.4	2.7	19.6					0.66	0.13	2.39	1.0
TNM stage	I	13	2	27.1	7.4	0.9	26.7	1.00				1.00			
	II	23	13	37.9	34.3	18.3	58.7	4.58	1.03	20.37	0.05	4.55	1.25	24.56	0.02
	III	20	4	44.8	8.9	2.4	22.9	1.20	0.22	6.53	0.8	4.69	0.94	29.13	0.06
	IVa–c†	133	36	268.2	13.4	9.4	18.6	1.72	0.41	7.16	0.5	9.26	2.41	52.15	0.001
	Missing	5	0	14.8	0.0	0.0	24.9					0.61	0.00	12.65	0.8
ICON–S stage§	I	16	4	32.0	12.5	3.4	32.1	1.00							
	II	71	7	155.1	4.5	1.8	9.3	0.37	0.11	1.26	0.1				
	III–IV‡	36	9	73.1	12.3	5.6	23.4	0.92	0.28	3.07	0.9				
	Missing	1	0	3.0	0.0	0.0	122.9								

CI, confidence interval; HR-HPV, high-risk human papillomavirus; ICON–S, International Collaboration on Oropharyngeal Cancer Network for Staging; SIMD, Scottish Index of Multiple Deprivation.

* Adjusted for all variables in the table except for ICON stage. They are highly associated with TNM stage and therefore not included in the adjusted model to avoid collinearity.

† For TNM stage, IVa–c combines the levels IVa (126 cases, 34 died/recur), IVb (6 cases, 2 died/recur) and IVc (1 case, 0 died/recur).

‡ For ICON stage, III–IV combines the levels III (35 cases, 9 died/recur) and IV (1 case, 0 died/recur).

§ Only select HR-HPV-positive cases, $n = 124$.

distribution of discordant results in OPC cases. Furthermore, HPV status determined by this assay has been shown to be independently associated with disease-specific survival, as indicated in previous studies, the national audit and the data outlined in the present manuscript [10,18]. Thus, although testing with multiple markers/chemistries for HPV status provides further validation, we are confident that the assay is a robust and credible marker of HPV status. Additionally, the advantage of this approach is that it also provides information on type-specific prevalence to allow the monitoring of epidemiological trends.

Country-specific data on the attributable fraction of HPV-associated disease are important as they will inform the decisions surrounding the utility and cost-effectiveness of HPV vaccination, including gender neutral vaccination. Certainly, a significant impact of the vaccine on HR-HPV prevalence and disease outcomes, including high-grade cervical intraepithelial neoplasia (CIN2+), has already been observed in young women attending for screening in Scotland [23,24]. As reports suggest that HPV vaccination induces HPV antibodies in the oral cavity it is reasonable to assume that the vaccine will ultimately have an impact on OPC incidence [25,26]. This may be realised more rapidly in a gender neutral programme, given that we and others have shown a significantly higher prevalence of OPCs associated with HPV in men compared with women [9].

We report an inverse association between HPV positivity and excess alcohol consumption. No other factor, including social deprivation or cancer stage, was associated with viral status. This is an interesting and important observation, as it indicates that in Scotland, patient-related demographic and clinical factors cannot be reliably used to predict the HPV status of patients' OPCs. Furthermore, we did not observe an inverse relationship between HPV positivity and smoking status, unlike other studies [9]. This may relate to high smoking rates and the socioeconomic status in our study population, but we acknowledge the limitation in the smoking status data we had available to us and it will be important in future studies to ensure the collection of a more detailed smoking history.

In our data, TNM classification 7 was more predictive of outcome than the new staging system that takes into account HPV status, although numbers were small. The British Association of Head and Neck Oncologists (BAHNO) has raised concerns of the use of TNM 8 and have suggested that TNM 7 continues to be recorded. Although the new staging system reflects improved understanding of cancer biology and the clinical outcome of HPV-positive OPCs, further research is required to define if TNM 8 is generalisable to all populations and can be used to change treatment decisions.

In the adjusted analysis, HPV status was strongly associated with an improvement in overall and PFS during a median of 2 years of follow-up. For individuals whose disease recurred, the median time to progression was similar for HPV-positive and HPV-negative OPCs, with most people experiencing disease progression in the first year after the completion of therapy [27]. The improvement in survival associated with HPV-positive disease is in accordance with other studies of OPC; D'Souza *et al.* [28] recently reported a 66% reduction in the hazard of death among HPV-positive OPCs compared with HPV-negative cases and in other HPV-associated cancers [29,30]. It has been hypothesised that improved prognosis in HPV-positive cancers may be due to better response rates to treatment through increased sensitivity to radiation and host immune response to the tumour [31]. Although treatment for HPV-positive and -negative OPCs remains the same in many settings, the results of de-intensifying treatment trials in HPV-positive OPC will be informative for future evidence-based management strategies [32]. The reduction in survival with increasing age and heavy alcohol consumption has implications for the ability to deliver and tolerate curative treatment, which may affect survival. Cause of death was out with the scope of the present study, but nutritional factors and co-morbidities in these patient groups should be further evaluated.

Conclusion

In this evaluation of OPC patients in whom the prevalence of traditional risk factors of drinking and smoking was high, 60% of tumours were HPV positive with positivity dominated by HPV type 16. Although we confirmed that in our population HR-HPV status was strongly associated with improved overall and PFS, HPV status could not be reliably predicted by demographic/clinical characteristics alone, emphasising the need for laboratory confirmation. Although primary prevention of most OPCs through vaccination will probably exert a significant influence on the burden of this disease in time, in the shorter term, Scotland must address other interventions to promote healthy living in order to reduce excessive alcohol consumption and smoking. Such interventions, if successfully implemented, would have far-reaching benefits beyond the reduction of OPC.

Conflict of Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2019.06.002.

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