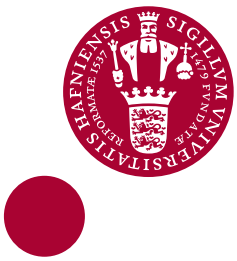

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ORIGINAL ARTICLE

Risk profiling based on p16 and HPV DNA more accurately predicts location of disease relapse in patients with oropharyngeal squamous cell carcinoma

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Background: In the era of precision medicine and HPV-related oropharyngeal squamous cell carcinoma (OPSCC), it is relevant to assess the risk of not only survival, but also the risk of local, regional, or distant treatment failure. The UICC 8th edition uses the surrogate marker p16 to stratify for HPV association but discordance between p16 status and HPV association has been shown. The purpose of this study was to develop a prognostic model to predict the risk of local, regional, and distant metastases and non-cancer-related death for patients with OPSCC, test the prognostic relevance of adding HPV DNA and p16 status, and validate the findings in an independent external dataset.

Patients and methods: Consecutive patients diagnosed with OPSCC and treated with curative radiotherapy with or without cisplatin in eastern Denmark from 2000 to 2014 were included. Characteristics included age, gender, TNM stage, smoking habits, performance status, and HPV status assessed with p16 and HPV DNA. The information was used to develop a prognostic model for first site of failure with four competing events: recurrence in T-, N-, and M-site, and death with no evidence of disease.

Results: Overall 1243 patients were eligible for the analysis. A prognostic model with the four events was developed and externally validated in an independent dataset with a heterogeneously treated patient population from another institution. The individual prognostication from the competing risk analysis is displayed in a user friendly online tool (https://rasmussen.shinyapps.io/OPSCCmodelHPV_p16/). Replacing p16 status with the combined variable HPV/p16 status influenced the HR and patients with HPV−/p16+ had significantly higher HR of M-site recurrence than HPV+/p16+ with a HR = 2.56; CI [1.30; 5.02]; $P = 0.006$ ($P = 0.013$ in the validation cohort).

Conclusion: Patients with HPV−/p16+ have significantly higher risk of M-site recurrence and could potentially be relevant candidates for clinical trials testing systemic treatments in combination with conventional treatments.

Key words: oropharyngeal squamous cell carcinoma, competing risk, prognostic model, UICC, HPV DNA

Introduction

In the era of precision medicine [1] and human papilloma virus (HPV)-related [2, 3] head and neck squamous cell carcinoma (HNSCC), it is relevant to assess the risk of not only survival, but also the distribution of risk for local, regional, and distant recurrence. The UICC/AJCC classification is developed with overall survival as sole end point, but the risk of T-, N-, and M-site recurrence is important information for patient counselling and trial

design with regards to local, regional, or systemic treatment intensification or de-intensification. Although the UICC/AJCC staging system is a prognostic tool and fulfills the four parameters to achieve a robust staging system, reviewed by Groome [4], it was never intended as a treatment guideline [5]. Although overall survival is a robust and meaningful measure, it may not be the most optimal end point to evaluate the effect of loco-regional treatments such as surgery and radiotherapy. Loco-regional failure is thus a common primary end point in clinical trials [6]. Loco-regional failure combines T- and N-site recurrence as a pragmatic measure, but the mortality and morbidity of T- or N-site failure differs as does the prognosis and the sequela after salvage treatment.

Complex prognostic models can aid clinicians and be used to ask and test relevant clinical questions or conundrums [7]. Even though several complex prognostic models with a variety of different prognosticators have been suggested, none have generally been accepted beyond the UICC/AJCC staging system [8]. This is mainly due to the challenge of proper validation [9, 10]. Regardless of the complexity, prognostic tools with only one end point is an oversimplification.

The UICC 8th edition uses the surrogate marker p16 to stratify for HPV association but there is evidence suggesting that the combination of p16 staining and HPV DNA is more accurate [11–14]. Both p16 status and HPV DNA have shown separately to be good prognostic factors, but a recent meta-analysis have reported less favorable prognosis for patients with HPV DNA negative but p16 positive tumors [14]. However, the prognostic relevance of combining p16 staining and HPV DNA in a prognostic model with multiple end points is currently unknown. Currently several trials testing de-intensification in patients with HPV associated tumors are ongoing [15] and in such de-intensification trials, selection of appropriate patients are critical.

The purpose of this study was threefold: (i) To develop a prognostic model with multiple end points (from now on prognostic model) that can guide clinicians in therapeutic decisions and identify patients for clinical trials testing treatment intensification or de-intensification; (ii) test the prognostic relevance of adding HPV DNA to p16 status in such a prognostic model; and (iii) Validate the findings from the prognostic model both with and without HPV DNA in an independent external dataset with a heterogeneously treated patient population.

Material and methods

Patients

The patient cohort used to develop the prognostic model is population based and includes patients diagnosed with oropharyngeal squamous cell carcinoma (OPSCC) in eastern Denmark from 2000 to 2014 [16]. Patients not treated with curative intent with radiotherapy with or without concomitant cisplatin were excluded up front as where patients with M1 disease.

Patient and tumor characteristics including age, gender, TNM stage, smoking habits, performance status (PS) (WHO), HPV status, and follow-up were retrieved, and all patients were restaged to 8th edition of UICC staging. In p16 negative patients, N2a and N2b were merged to N2, and N2c and N3 was merged to N3*. HPV status of tumor tissue was assessed with both p16 immunostaining scored according to EORTC/DAHANCA guidelines [17] and with detection of HPV DNA as were explained in details elsewhere [18]. The information was used to develop a prognostic model for first site of failure with four competing events: recurrence in T-, N-, and M-site or death with no evidence of disease (death NED).

The validation cohort consists of patients with OPSCC treated at the Department of Oto-Rhino-Laryngology, Head and Neck Surgery of the University of Giessen, Germany from 2000 to 2009 [11]. The patients in the validation cohort were treated more heterogeneously with surgery and/or radiotherapy depending on stage and patient preference [11]. We allow flexibility to account for a difference in baseline risk between training and validation set, for example due to case-mix and the different treatment options, by focusing on comparison of hazard ratios only using a model updating strategy [19]. In other words, we allow the absolute risks to differ, but compare hazard ratios for the identified covariables for each end point.

Statistics

Four cause-specific Cox regression models were built for the hazard rates of recurrence in T-, N-, M-site, and death NED, respectively. The four events were defined as follows:

- T-site recurrence: T-site recurrence or simultaneous T- and N-site recurrence.
- N-site recurrence: N-site recurrence
- M-site recurrence: M-site recurrence, T- and/or N-site and M-site recurrence.
- Death NED: death with no evidence of prior recurrence.

For all four events, the following variables were included in the model, referred to as p16 model:

- Gender, age, PS, smoking habits, p16 status, T-, and N-stage.

The six variables were chosen before the analysis was conducted and were chosen based on clinical experience, relevance for the four outcomes and accessibility. To retain simplicity the same variables were used in all four end points [20], and no interaction terms were included because of limited number of events for some end points [21].

Time was measured from time of diagnosis to an event or last follow-up and the median follow-up time was estimated using the reverse Kaplan–Meier method [22]. Overall absolute risks for the four events were estimated using the Aalen–Johansen method and difference in absolute risk was tested with Gray's test. Individual risk predictions after 2 and 5 years were assessed for any given patient by combing the four cause-specific Cox regression models [23] in a competing risk analysis. Risk estimates were displayed in absolute risks in an online tool allowing variation of baseline characteristics and risk profiling for an example patient. Description of assumption testing and imputation of missing values are provided in the supplementary material, available at *Annals of Oncology* online.

The relevance of adding HPV DNA status in a prognostic model was assessed as follows: four groups related to HPV/p16 were created (HPV+/p16+; HPV+/p16−; HPV−/p16+; and HPV−/p16−) and entered in the p16 model as a categorical variable yielding a model, referred to as the HPV/p16 model. The performance of the p16 and the HPV/p16 model was compared using the Brier score as well as a time-dependent concordance index [24] after 5 years corresponding to an area under the curve (AUC) where 1 corresponds to the perfect model and 0.5 corresponds to a coin toss.

The HR from the four cause-specific models were validated by applying both the model with and without HPV DNA to an external validation cohort. Overall survival *after* T-, N-, and M-site recurrence were plotted using the Kaplan–Meier product limit estimator.

All statistical analysis was performed in the software R using the packages survival, timereg, and riskRegression.

Results

Overall 1541 patients were diagnosed with OSPCC from 2000 to 2014 in eastern Denmark. After exclusion of patients not treated with curative intent, 1243 patients were eligible for further analysis. Patient characteristics can be seen from Table 1.

Median follow-up was 7.2 years. Of the 1243 included patients, 100 patients (8.0%) experienced T-site recurrence, 97 patients (7.8%) experienced N-site recurrence, 91 patients (7.3%) experienced M-site recurrence, and 267 patients (21.5%) died with NED. The remaining 679 patients (55.0%) had not experienced any failure at last follow-up.

Figure 1 depicts the absolute risk stratified for p16 and HPV DNA status combined. Supplementary Figure S1, available at *Annals of Oncology* online depicts the absolute risk stratified by p16 status and HPV DNA status separately.

As expected, patients with HPV negative tumors had a higher risk for all four events than patients with HPV positive tumors assessed with p16 or with HPV DNA detection. Patients with HPV−/p16+ tumors have a high 5-year risk of M-site recurrence [14.8% (95% CI: 6.7% to 23.0%)] compared to patients with other combinations of p16 and HPV DNA status [6.6% (4.6% to 8.5%) for HPV+/p16+ patients, 4.9% (0.0% to 11.5%) for HPV+/p16− patients and 9.0% (6.0% to 11.9%) for HPV−/p16− patients]. The absolute risk of M-site recurrence was significantly higher for patients with HPV−/p16+ ($P = 0.03$, tested with Gray's test).

Figure 2 depicts the survival after T-, N-, and M-site recurrence respectively stratified by p16 status and HPV DNA. Failure site influenced survival and there was a significant difference in survival between patients with HPV negative and HPV positive tumors assessed with both p16 status and HPV DNA for all three failure sites. As illustrated in Figures 1 and 2, there was discordance in HPV assessment by p16 and by HPV DNA detection in 120 patients (9.7%) (Table 1).

In the p16 model, T-stage and p16 status were significant predictors for T-site recurrence. N-stage and p16 status were significant predictors for N-site recurrence. PS, smoking habits (>20 pack years), T-stage and N-stage were significant predictors for M-site recurrence. Gender, age, PS, p16 status, T-, and N-stage were significant predictors and smoking habits was borderline significant for death NED (Table 2). The individual prognostication of absolute risks of the four events after 2 and 5 years for any given patient based on the six known characteristics and p16 status can be assessed in an online tool (https://rasmussen.shinyapps.io/OPSCCmodel/) (p16 model).

Replacing p16 status with the combined variable HPV/p16 status influenced the HR in the HPV/p16 model (Table 2). Interestingly, patients with HPV−/p16+ had significantly higher HR of M-site recurrence than HPV+/p16+ with a HR = 2.56; CI [1.30; 5.02]; $P = 0.006$. Whereas patients with HPV+/p16− and HPV−/p16− did not with a HR = 0.46 ($P = 0.287$) and HR = 0.93 ($P = 0.797$), respectively. Moreover, only patients negative for HPV DNA had a significantly higher risk of T- or N-

Table 1. Patient characteristics for the two patient cohorts

Characteristics	Level	No. (%)	
		Test cohort, eastern Denmark (n = 1243)	Validation cohort, Giessen, Germany (n = 339)
Gender	Female	340 (27.4)	74 (21.8)
	Male	903 (72.6)	265 (78.2)
Age	Mean (SD)	60.2 (9.3)	59.4 (9.4)
PS	0	946 (76.1)	237 (69.9)
	1–3	297 (23.9)	102 (30.1)
Smoking habits in pack years	0	280 (22.5)	37 (10.9)
	0–10	100 (8.0)	16 (4.7)
	10–20	119 (9.6)	25 (7.4)
	>20	744 (59.9)	261 (77.0)
P16 status	Positive	822 (66.1)	85 (25.1)
	Negative	421 (33.9)	254 (74.9)
HPV/p16	+/+	745 (59.9)	61 (18.0)
	+/−	43 (3.5)	22 (6.5)
	−/+	77 (6.2)	24 (7.1)
	−/−	378 (30.4)	232 (68.4)
T-stage	1	270 (21.7)	76 (22.4)
	2	577 (46.4)	102 (30.1)
	3	283 (22.8)	78 (23.0)
	4	113 (9.1)	83 (24.5)
N-stage	0	263 (21.2)	98 (28.9)
	1	659 (53.0)	82 (24.2)
	2	213 (17.1)	104 (30.7)
	3*	108 (8.7)	55 (16.2)

In p16 negative patientsN2c and N3 was merged into N3*.

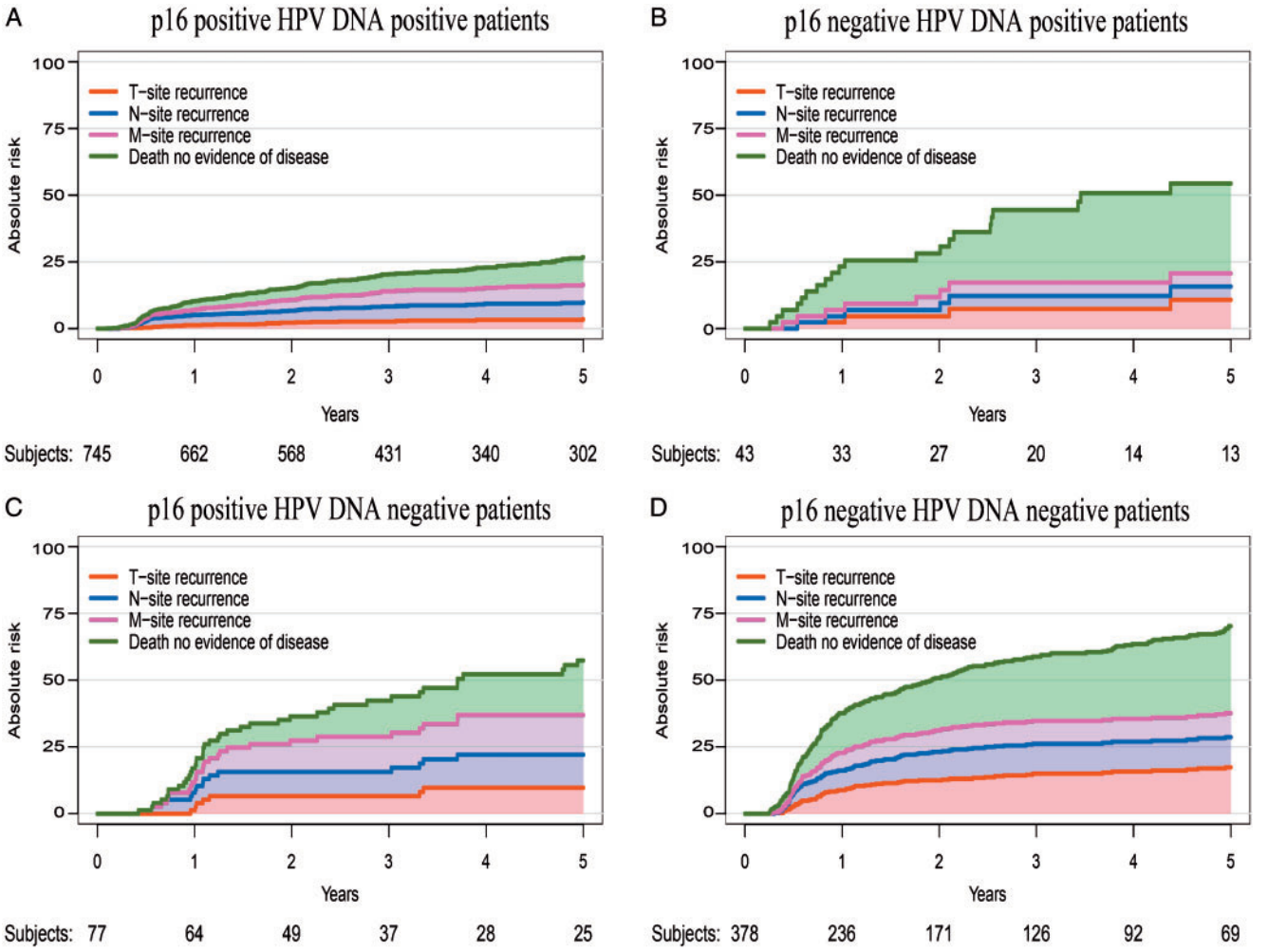

Figure 1. Overall absolute risks of T-site, N-site, M-site recurrence, and death NED for p16 positive HPV DNA positive patients (A), p16 negative HPV DNA positive patients (B), p16 positive HPV DNA negative patients (C), and p16 negative HPV DNA negative patients (D). The red curve depicts the absolute risk of T-site recurrence, the blue curve depicts the absolute risk N-site recurrence, the gray curve depicts the absolute risk of M-site recurrence, and the green curve depicts the absolute risk of death no evidence of disease.

site recurrence regardless of p16 status. All combinations of HPV DNA status and p16 status were significant predictors for death NED. Individual prognostication with absolute risk prediction for patient characteristics including the combination of HPV DNA and p16 status can be assessed in the online tool (https://rasmussen.shinyapps.io/OPSCCmodelHPV_p16/) (HPV/p16 model).

The p16 model had an AUC of 75.2, 62.9, 71.8, and 73.4 for T-, N, M-site recurrence, and death NED, respectively, while the HPV/p16 model which had an AUC of 75.6, 65.0, 72.4, 73.6 for the four different events. There was no statistically difference in performance for any of the four events between the p16 model and the HPV/p16 model tested with AUC and Brier score ($P > 0.1$). Of note, AUC and Brier score looks at mean performance for the whole population. The performance for the 120 patients with discordance in HPV assessment could be clinically very relevant.

The p16 and p16/HPV models were refitted in the external dataset and the resulting HR's compared for validation. Patient characteristics for the validation cohort can be seen in Table 1 and the patient cohort has previously been described [11, 25]. Median follow-up was 9.2 years. Of the 339 included patients, 56 patients experienced T-site recurrence, 3 patients experienced N-site recurrence, 29 patients experienced M-site recurrence, and 149 patients died with NED. Due to very few patients with N-site recurrence, it was not possible to consider this end point. In the external cohort, HPV−/p16+ patients also had a significantly higher HR than HPV+/p16+ patients of M-site recurrence with a point estimate of HR = 16.6; CI [1.8; 153]; $P = 0.013$ (supplementary Table S2, available at *Annals of Oncology* online). However, the absolute risk for M-site recurrence for HPV−/p16+ was not statistically significant difference tested with Gray's test ($P = 0.07$). The results can be seen in supplementary Table S2, available at *Annals of Oncology* online.

Discussion

This is the first study to develop a prognostic model for recurrence in T-, N- or M-site, and death NED for head and neck cancer and the result is presented in a user friendly and readable

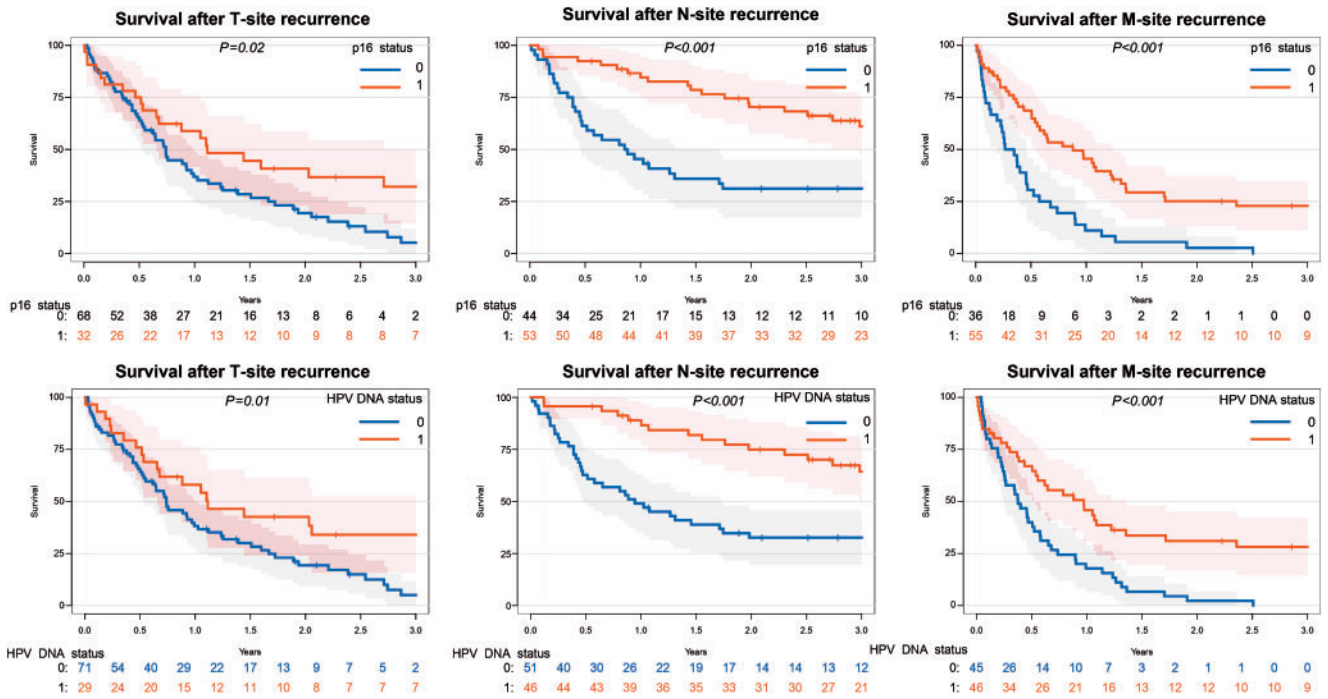

Figure 2. Survival curve illustrating survival in the test cohort after T-, N-, and M-site recurrence respectively stratified by p16 status (top panel) and by HPV DNA (bottom panel) (0 = negative and 1 positive).

online tool (https://rasmussen.shinyapps.io/OPSCCmodelHPV_p16/) that can support the clinical decision process. Combining HPV DNA detection with p16 showed that HPV−/p16+ was a significant predictor for M-site recurrence and patients with HPV−/p16+ tumors had a significantly higher HR for M-site recurrence in both patient cohorts. The risk of M-site recurrence doubled for patients with HPV−/p16+ tumors compared with the other combinations of HPV DNA and p16 status. A male 55-year-old patient with T2N2 disease, PS 0 and 10 pack years will with a HPV+/p16+ tumor have an absolute risk of M-site recurrence of 5.5% after 5 years, whereas a patient with similar characteristics but a HPV−/p16+ tumor will have an absolute risk of 11.5% for M-site recurrence after 5 years (https://rasmussen.shinyapps.io/OPSCCmodelHPV_p16/). To our knowledge, a higher risk of M-site failures for HPV−/p16+ patients has previously not been reported. The existing literature have mainly focused on overall survival and not on specific failure sites. However, one study [26] did report a significantly lower risk of distant metastases in patients whose tumors had a high expression of p53. Both p16 and p53 are important tumor suppressor proteins and one study reported that p16 and p53 formed a heteromeric complex involved in physiological functions [27]. Such effects might be an explanation of increased risk of M-site failure as observed here. Nevertheless, it remains speculation and the underlying biologic mechanism for the increased risk of M-site failure is currently unknown.

Regardless of biologic cause, patients with HPV−/p16+ could potentially be relevant candidates for clinical trials testing systemic treatments in combination with conventional treatment such as induction chemotherapy or other systemic treatment approaches. The online application could be used to identify relevant patients eligible for such trials. Albeit the HR's was significant in both cohorts and the difference in absolute risk was significant in the test cohort, the difference in absolute risk was not statistically different in the validation cohort. As such the results should be validated further in other patient cohorts.

The identification of relevant eligible patients is critical in both escalation and de-escalation trials and nomograms are usually proposed or used as tool to risk stratify patients [16]. Albeit nomograms provide illustration that can augment patient communication, they can be difficult to read and do not provide uncertainty of the estimate. The online tool presented here arguably has an advantage in ease of use and provides confidence intervals for all estimates. Such online tools can be externally validated through absolute risk prediction and we propose that externally validated, site-specific models would be a tangible improvement over current prognostic models [20].

The site of recurrence influences both mortality and morbidity in patients with OPSCC, illustrating the importance to distinguish between recurrence sites to individualize patients' prognosis (Figure 2). UICC/AJCC classification is developed and validated with OS as sole end point and there is a risk of including patients with a high risk of death NED, who might not benefit from intensified treatment. Since both surgery and radiotherapy are loco-regional treatments, the same argument can be stated for patients in high risk of M-site recurrence who intuitively seem to be more relevant candidates for trials testing systemic treatments. Equally, in de-escalation trials it is desirable to include only patients with a good prognosis. Here, it should be kept in mind that UICC was never intended as a clinical treatment guideline and it is probably too simplistic for use as inclusion criterion. Individualized prognostication focusing on more than survival is not only relevant for the patient but is a necessity for trial designs aiming at precision medicine or individualized treatment.

Table 2. The four cause-specific cox models for the p16 model and the HPV/p16 model

		p16 model			HPV/p16 model		
Characteristics	**Level**	**Hazard ratio**	**Confidence interval**	***P*-value**	**Hazard ratio**	**Confidence interval**	***P*-value**
T-site recurrence							
Gender	Female	1.00	(reference)	–	1.00	(reference)	–
	Male	1.37	[0.86; 2.18]	0.191	1.41	[0.88; 2.24]	0.159
Age	Increase in year	0.99	[0.97; 1.02]	0.490	1.40	[0.97; 1.01]	0.386
PS	0	1.00	(reference)	–	1.00	(reference)	–
	1–3	1.47	[0.96; 2.25]	0.080	1.45	[0.94; 2.22]	0.091
Smoking habits in pack years	0	1.00	(reference)	–	1.00	(reference)	–
	0–10	1.90	[0.67; 5.37]	0.227	1.67	[0.59; 4.75]	0.337
	10–20	0.66	[0.20; 2.16]	0.487	0.61	[0.19; 2.01]	0.416
	>20	1.66	[0.80; 3.43]	0.175	1.50	[0.72; 3.13]	0.281
P16 status	Negative	1.00	(reference)	–			
	Positive	0.27	[0.16; 0.43]	<0.001*			
HPV/p16	1 (+/+)				1.00	(reference)	–
	2 (+/−)				2.52	[0.86; 7.33]	0.091
	3 (−/+)				2.75	[1.18; 6.43]	0.019*
	4 (−/−)				4.78	[2.81; 8.12]	<0.001*
T-stage	Increase in T-stage	1.78	[1.42; 2.24]	<0.001*	1.75	[1.38; 2.20]	<0.001*
N-stage	Increase in N-stage	0.97	[0.79; 1.19]	0.786	0.98	[0.80; 1.19]	0.808
N-site recurrence							
Gender	Female	1.00	(reference)	–	1.00	(reference)	–
	Male	1.59	[0.96; 2.64]	0.072	1.64	[0.99; 2.18]	0.057
Age	Increase in year	0.99	[0.96; 1.01]	0.235	0.98	[0.96; 1.01]	0.179
PS	0	1.00	(reference)	–	1.00	(reference)	–
	1–3	1.04	[0.63; 1.72]	0.8768	1.01	[0.61; 1.68]	0.955
Smoking habits in pack years	0	1.00	(reference)	–	1.00	(reference)	–
	0–10	0.77	[0.31; 1.93]	0.581	0.68	[0.27; 1.72]	0.416
	10–20	0.78	[0.34; 1.80]	0.566	0.72	[0.31; 1.66]	0.441
	>20	0.93	[0.54; 1.61]	0.803	0.83	[0.47; 1.45]	0.506
p16 status	Negative	1.00	(reference)	–			
	Positive	0.55	[0.34; 0.89]	0.015*			
HPV/p16	1 (+/+)				1.00	(reference)	–
	2 (+/−)				0.73	[0.18; 3.05]	0.668
	3 (−/+)				2.34	[1.13; 4.86]	0.023*
	4 (−/−)				2.32	[1.39; 3.89]	0.001*
T-stage	Increase in T-stage	1.05	[0.83; 1.34]	0.666	1.04	[0.82; 1.31]	0.769
N-stage	Increase in N-stage	1.55	[1.25; 1.93]	<0.001*	1.56	[1.25; 1.94]	<0.001*
M-site recurrence							
Gender	Female	1.00	(reference)	–	1.00	(reference)	–
	Male	1.49	[0.88; 2.52]	0.139	1.49	[0.89; 2.52]	0.139
Age	Increase in year	0.99	[0.97; 1.01]	0.404	0.99	[0.96; 1.01]	0.257
PS	0	1.00	(reference)	–	1.00	(reference)	–
	1–3	2.06	[1.29; 3.28]	0.002*	2.04	[1.28; 3.25]	0.003*
Smoking habits in pack years	0	1.00	(reference)	–	1.00	(reference)	–
	0–10	1.00	[0.26; 3.78]	0.999	0.86	[0.23; 3.28]	0.838
	10–20	1.30	[0.42; 3.99]	0.647	1.22	[0.40; 3.75]	0.728
	>20	3.14	[1.48; 6.64]	0.002*	2.88	[1.35; 6.12]	0.006*
P16 status	Negative	1.00	(reference)	–			
	Positive	1.32	[0.81; 2.14]	0.262			
HPV/p16	1 (+/+)				1.00	(reference)	–
	2 (+/−)				0.46	[0.11; 1.92]	0.287
	3 (−/+)				2.56	[1.30; 5.02]	0.006*
	4 (−/−)				0.93	[0.56; 1.56]	0.797
T-stage	Increase in T-stage	1.33	[1.05; 1.70]	0.020*	1.31	[1.03; 1.66]	0.028*
N-stage	Increase in N-stage	1.90	[1.52; 2.39]	<0.001*	1.92	[1.53; 2.42]	<0.001*

Continued

Table 2. *Continued*

Characteristics	Level	p16 model			HPV/p16 model		
		Hazard ratio	Confidence interval	*P*-value	Hazard ratio	Confidence interval	*P*-value
Death NED							
Gender	Female	1.00	(reference)	–	1.00	(reference)	–
	Male	1.52	[1.14; 2.01]	0.004*	1.52	[1.14; 2.01]	0.004*
Age	Increase in year	1.06	[1.04; 1.07]	<0.001*	1.06	[1.04; 1.07]	<0.001*
PS	0	1.00	(reference)	–	1.00	(reference)	–
	1–3	1.71	[1.31; 2.24]	<0.001*	1.71	[1.31; 2.24]	<0.001*
Smoking habits in pack years	0	1.00	(reference)	–	1.00	(reference)	–
	0–10	0.85	[0.44; 1.62]	0.617	0.84	[0.44; 1.59]	0.585
	10–20	0.89	[0.51; 1.54]	0.669	0.88	[0.51; 1.54]	0.656
	>20	1.45	[0.99; 2.14]	0.057	1.44	[0.96; 2.08]	0.082
P16 status	Negative	1.00	(reference)	–			
	Positive	0.36	[0.27; 0.47]	<0.001*			
HPV/p16	1 (+/+)				1.00	(reference)	–
	2 (+/−)				3.06	[1.78; 5.24]	<0.001*
	3 (−/+)				1.92	[1.17; 3.14]	0.010*
	4 (−/−)				3.07	[2.27; 4.14]	<0.001*
T-stage	Increase in T-stage	1.39	[1.20; 1.60]	<0.001*	1.38	[1.20; 1.60]	<0.001*
N-stage	Increase in N-stage	1.25	[1.10; 1.42]	<0.001*	1.25	[1.10; 1.43]	<0.001*

*Significant *P*-values.

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Disclosure

The authors have declared no conflicts of interest.

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