Nodal response after 46 Gy of intensity-modulated radiotherapy is associated with human papillomavirus-related oropharyngeal carcinoma.

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ABSTRACT

This study aimed to analyze the effect of human papillomavirus associated T1-2 node positive oropharyngeal carcinoma (HPV+ OPSCC) on nodal response, recurrent disease and survival in patients treated according to the Rotterdam protocol. In total 77 patients with T1-2 OPSCC with nodal disease, treated between 2000-2012, were included in this study. Patients were treated according to 'the Rotterdam protocol': 46 Gy of IMRT followed by a local boost using cyberknife or brachytherapy (22 Gy) and neck dissection. The presence of HPV was determined by p16 INK4A immunostaining. Outcomes were overall survival, disease free survival and the extent of nodal response. Nodal stage was determined following 7th and 8th AJCC/UICC classification. 68.4% of patients had p16 positive disease. 35.4% of all patients achieved complete nodal response (pN0) after 46 Gy of IMRT. Based on the 7th TNM classification, nodal response (partial or complete) was significantly associated with HPV status (p=0.002). Patients with p16-positive OPSCC had an OR of 4.6 to achieve complete nodal response. However, smoking interacted with this effect. Applying the 8th TNM classification, complete or partial response was associated with HPV status, however not significant (OR 1.7, p=0.138). Complete nodal response lead to 100% overall survival in p16-positive OPSCC. HPV-related OPSCC are associated with complete nodal response after 46 Gy of IMRT. Patients with full regional control (pN0) after IMRT and subsequent neck dissection show a significantly better overall survival, but smoking negatively interacts with this effect.

INTRODUCTION

Currently, over 70% of oropharyngeal squamous cell carcinoma (OPSCC) in Europe is associated with Human Papilloma Virus (HPV).[1] Patients with HPV-positive OPSCC tend to be young and fit at presentation. Also, HPV-positive OPSCC has a 58% reduction in the risk of death compared to HPV-negative OPSCC.[2] A common presentation of HPV-related OPSCC is that of an early primary tumor (T1-2) along with advanced nodal disease (N2-3).[3,4] Disease control rates for patients with HPV-positive OPSCC are significantly better than that seen in HPV-negative OPSCC. Several studies have also shown that HPV associated OPSCC is more radio-sensitive than HPV-negative OPSCC.[2,5]

Due to their advanced nodal disease, patients with HPV-positive OPSCC are considered advanced stage (III-IVb) disease. Therefore patients with HPV-positive disease traditionally are treated with intensive multimodality regimens. However, the reality is that their outlook is actually more favorable.[2] Therefore, recently the 8th edition UICC/AJCC TNM staging of OPSCC has been divided into two different staging systems for both HPV associated (p16 positive) OPSCC and non-HPV associated (p16 negative) OPSCC. [6] This staging system specifically results in a change of nodal stage categories, both clinical and pathological, and represent a significant change for HPV-positive OPSCC from the non-HPV associated OPSCC. [7] Hence, it permits a more appropriate depiction of the prognosis of HPV-positive disease than is supplied by the 7th edition TNM classification.[8]

The optimal treatment of nodal disease in (HPV-positive) OPSCC has therefore become controversial. Planned neck dissection following definitive radiotherapy has been considered standard of care in the past. However, data suggest that patients with a complete clinical and radiographic response to primary nonsurgical treatment can be observed without neck dissection and followed with imaging studies.[2,9] Some institutions do not perform neck dissection if a complete response of the neck is achieved after radiotherapy (70Gy), regardless of the original size of the metastasis. Other studies recommend neck dissection in patients with N2-N3 disease regardless of response to the oncologic treatment, but also show an improved disease-free survival.[10-13] This leads to the fundamental question if and when to perform a neck dissection in patients with OPSCC. In addition to this question, several clinical trials, such as ECOG 311 and Pathos, are currently investigating de-intensification strategies, aiming to maintain a high cure rate while limiting short and longer term treatment related side effects.[14-16]

In 2000, 'the Rotterdam protocol' was introduced at our institution for patients with T1-2 OPSCC. This is an organ function preservation protocol and includes intensity modulated radiotherapy (IMRT) of 46 Gy to the primary tumor and the neck nodes in 23 fractions,

followed by a local boost using brachytherapy 22 Gy in 8 fractions (BT) or cyberknife 16.5 Gy in 3 fractions (CK) and a neck dissection in case of node positive (N+) disease. The local tumor is therefore treated with at least 66 Gy and nodal disease with 46 Gy. Patients with T3-4 tumors or advanced nodal disease (N3) receive concomitant chemoradiation with cisplatin.[17] Although two radiation techniques (IMRT and BT or CK) are used, the Rotterdam protocol aims for low toxicity and therefore could be considered in line with other de-intensification strategies.

We reported earlier that the Rotterdam protocol results in excellent local control rates compared to 46 Gy of 2-dimensional (2D) or 3D conformal radiotherapy followed by a BT boost, a technique we used from 1990 until 2000. In N+ disease, neck dissection after a relatively low dose of IMRT to the involved neck resulted in excellent regional control, because no regional failure was reported in those patients.[17] However, we did not collect HPV data on patients in this series. It is possible that the improved oncologic outcomes are not only attributable to the Rotterdam protocol, but also related to HPV-positive disease. Especially since an increased incidence of HPV-positive OPSCC was seen in the past decade compared to 1990 and 2000.

The objective of this study is therefore to describe the role of HPV status in patients with T1-2 node positive OPSCC, treated according to the Rotterdam protocol. Furthermore we want to analyze the effect of HPV-positive disease on nodal response, recurrent disease and survival in the study population, taking into account both 7th and 8th TNM classification. Finally we want to answer the earlier formulated question if and when to perform a neck dissection in patients with OPSCC, especially in case of smaller primary tumors with advanced nodal disease.

PATIENTS AND METHODS

This retrospective cohort study was conducted after approval was given by the institutional Medical Ethical Committee (MEC-2015-171). A waiver of informed consent was also given by the same ethics committee. Tissue samples were used and analyzed according to the FEDERA guidelines.

Patient demographics

Between 2000 and 2012, n=131 patients were identified who were treated according to the Rotterdam protocol for T1-T2 node positive OPSCC. Diagnosis of squamous cell carcinoma was confirmed according to histopathology; carcinoma in situ was excluded. Only patients of whom FFPE (formaldehyde fixed and paraffin embedded) pretreatment

samples were available in the archives of the Pathology department were included. Patients were excluded in case there was not enough previously untreated tissue sample left to perform HPV analysis. A total of n=77 patients remained for evaluation.

Demographic and clinical characteristics of patients were collected by a detailed medical chart review. Variables included were age, gender, comorbidity, clinical and pathological tumor- and nodal stage (both 7th and 8th UICC/AJCC TNM classification), extranodal extension (ENE), smoking habits, acute toxicity following radiotherapy, complications following surgery, survival status and cancer recurrence. Acute toxicity was scored based on chart reviews using the RTOG/EORTC criteria.[18] Complications following surgery were scored based on the Clavien-Dindo classification.[19]

Analysis of HPV

Immunohistochemical (IHC) analysis was performed for p16 INK4A. Strong and diffuse nuclear and cytoplasmic immunostaining in more than 70% of the tumor cells was considered as p16-positive. p16 staining is a well-established cost-effective surrogate for HPV status in oropharyngeal cancer compared with other methods (e.g. in-situ hybridisation or PCR), if scored and interpreted appropriately. [21-23]

Analysis of primary tumor and nodal disease

Tumor stage classification was determined according to both the 7th and the 8th UICC/AJCC TNM staging. Staging was performed by physical examination, CT or MRI, endoscopy and fine needle aspiration of pathological nodes, and/or biopsy of the primary tumor site. The histopathological examination of the neck dissection sample included identifying the number and location of the lymph nodes containing active metastatic disease, that is remaining viable tumor cells with presence of mitosis in tumor cells. The amount of viable tumor was estimated and percentage of viable tumor cell was given, which was correlated to the clinical response. Patients were considered to have complete nodal response in case no viable tumor cells were seen in their neck dissection sample (pN0). If viable tumor cells were identified in the neck dissection sample, but less lymph nodes were affected in comparison to the clinical TNM staging, patients were considered to have partial nodal response (pN < cN).

Statistical analysis

Data were analyzed with IBM SPSS Statistics 24.0 for Windows and R statistical software version 3.4.2. All tests were 2-sided with a significance level of 0.05. Univariate analysis of associations between categorical variables, HPV status and nodal status was done using Pearson chi-square tests. Multivariate analysis was performed by binary logistic regression analysis using complete or partial nodal response as outcome of interest. Overall survival (OS) was calculated from the date of diagnosis to the date of death. Disease free survival

(DFS) was defined as recurrent locoregional disease or distant metastasis. Survival was first examined using Kaplan-Meier univariate survival analysis followed by the log-rank test. Multivariate Cox Proportional Hazards Regression Analysis was then performed using OS and DFS as outcomes.

Table 1. Patient characteristics

		p16 negative			p16 positive		
		Mean (SD)	N	%	Mean (SD)	N	%
Age (years)		59 (8)			58 (9)		
Gender	Male		16	28.1%		41	71.9%
	Female		8	40.0%		12	60.0%
ACE27	None		8	26.7%		22	73.3%
	Mild		6	25.0%		18	75.0%
	moderate		9	47.4%		10	52.6%
	Severe		1	25.0%		3	75.0%
Tobacco use	No smoking history		0	0.0%		16	100.0%
	Former smoker		12	33.3%		24	66.7%
	Current smoker		12	48.0%		13	52.0%
Clinical T stage	T1		7	20.6%		27	79.4%
	T2		17	39.5%		26	60.5%
Clinical N stage (7th TNM classification)	N0		0	0.0%		0	0.0%
	N1		13	48.1%		14	51.9%
	N2a		2	14.3%		12	85.7%
	N2b		7	25.0%		21	75.0%
	N2c		1	16.7%		5	83.3%
	N3		1	50.0%		1	50.0%
Clinical N stage (8th TNM classification)	N0		0	0.0%		0	0.0%
	N1		13	21.7%		47	78.3%
	N2		0	0.0%		5	100.0%
	N3		0	0.0%		1	100.0%
	N2a		2	100.0%		0	0.0%
	N2b		5	100.0%		0	0.0%
	N2c		0	0.0%		0	0.0%
	N3a		1	100.0%		0	0.0%
	N3b		3	100.0%		0	0.0%
Extranodal extension	No		21	29.2%		51	70.8%
	Yes		3	60.0%		2	40.0%
Interval between last day of radiation and neck dissection (days)		14 (9)			14 (9)		
Recurrent disease	No recurrent disease		15	22.7%		51	77.3%
	(Loco)regional recurrence		5	100.0%		0	0.0%
	Distant metastasis		4	66.7%		2	33.3%
Deceased	No		11	19.0%		47	81.0%
	Yes		13	68.4%		6	31.6%
Follow up time (months)		54 (45)			69 (36)		

RESULTS

Patient characteristics

Table 1 shows the descriptive characteristics of the included patients. The majority of patients (68.8%) had p16-positive disease. Mean age at diagnosis was 58 years, and most patients were male (74.0%). Advanced disease was seen in over 70% of patients and the mean interval between last day of radiation therapy and day of neck dissection was 14 days (SD 9 days). Acute toxicity after radiation was low while 48.1% of patients had mild complications after neck dissection (Table 2).

Table 2. Acute toxicity after radiation and complications after neckdissection

Acute toxicity		
	N	%
grade 0	6	7.8%
grade 1	23	29.8%
Xerostomia	22	
Mucositis	1	
grade 2	37	48.1%
Xerostomia	2	
Mucositis	31	
Pain	4	
grade 3	8	10.4%
Dysphagia	6	
Pain	1	
Dyspnea	1	
grade 4	3	3.9%
Dyspnea	1	
ulceration of the skin	2	
Complications after neckdissection		
Grade 0 (no complications)	40	51.9%
Grade 1 (wound infections, hematoma, shoulder complaints requiring physiotherapy)	25	32.5%
Grade 2 (wound infections requiring antibiotics, wound dehiscence)	9	11.7%
Grade 3b (bleeding requiring revision surgery)	3	3.9%

Effect of the Rotterdam protocol for OPSCC on nodal response

Clinical nodal stage compared to pathological nodal stage after neck dissection resulted in 36.4% (n=28) of patients with complete nodal response (pN0) after 46 Gy of IMRT. The majority of the patients with pN0 necks, 82.1% (n=23), had p16-positive disease.

A proportion of patients had partial nodal response: 19.5% (n=15) using the 7th AJCC TNM classification and only 5.2% (n=4) using the 8th AJCC TNM classification. Using the 8th TNM classification the majority of patients did not have any significant change in their nodal disease after 46 Gy of IMRT (54.5%, n=42) and a minority of them showed a larger N-status after radiation (3.9%, n=3). Table 3 outlines the differences between cN an pN after IMRT, and the effect of p16-positive disease on nodal response while Figure 1 shows an overview of the differences in nodal response following tumor staging using the 7th and 8th TNM classification

Figure 1. UICC/AJCC Stage grouping, cN compared to pN after 46 Gy of IMRT

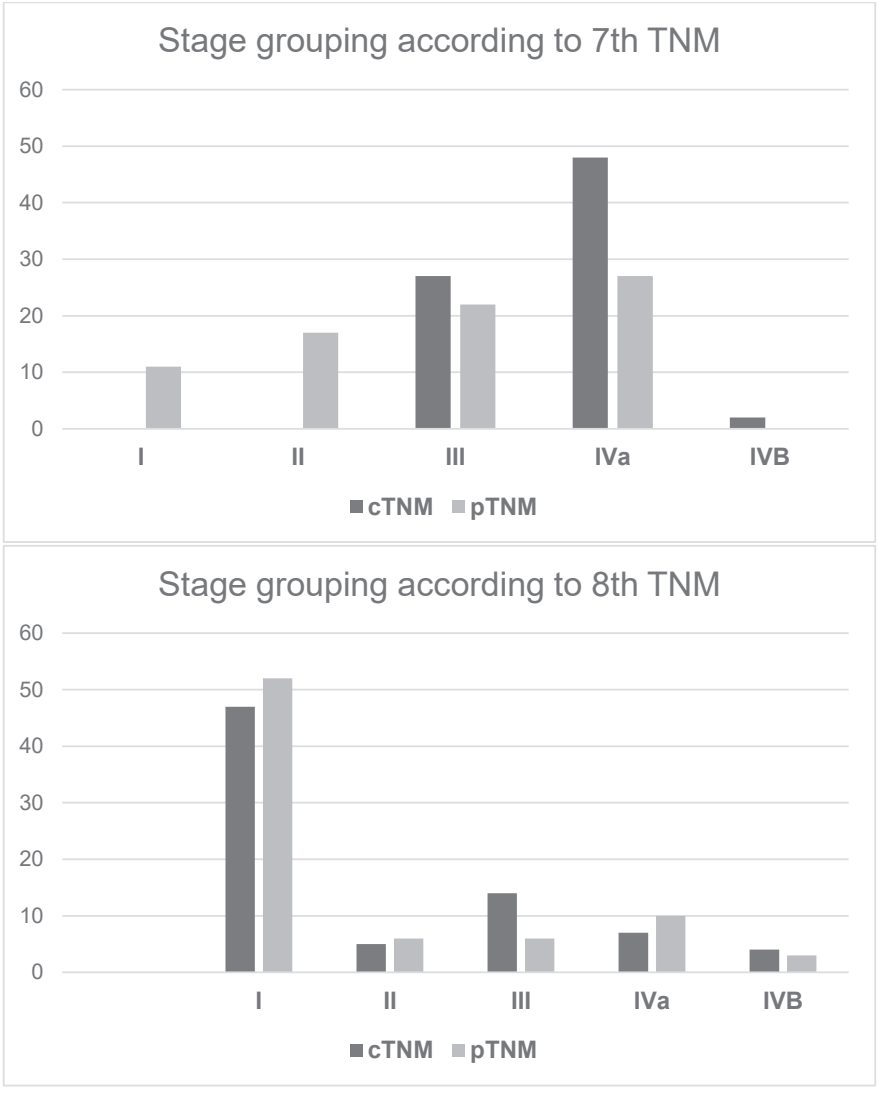


Table 3a. cN compared to pN after 46 Gy of IMRT using 7th TNM classification

p16 positive OPSCC – 7th TNM classification

Clinical nodal stage	*Pathological nodal stage (after neckdissection)*											
	pN0		**pN1**		**pN2a**		**pN2b**		**pN2c**		**Total**
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*
cN1	7†		5		0		0		0		12
cN2a	6†		4‡		0		0		0		10
cN2b	7†		6‡		1‡		11		0		25
cN2c	3†		1‡		0		0		1		5
cN3	0		0		0		1‡		0		1
Total	23	43.4%	16	30.2%	1	1.9%	12	22.6%	1	1.9%	53

† *Full nodal response (pathologically cancer free),* ‡*Partial nodal response (pN < cN)*

p16 negative OPSCC – 7th TNM classification

Clinical nodal stage	*Pathological nodal stage (after neckdissection)*											
	pN0		**pN1**		**pN2a**		**pN2b**		**pN2c**		**Total**
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*
cN1	5†		5		0		3§		0		13
cN2a	0		0		2		0		0		2
cN2b	0		1‡		0		6		0		7
cN2c	0		0		0		0		1		1
cN3	0		0		1‡		0		0		1
Total	5	20.8%	6	25%	3	12.5%	9	37.5%	1	4.2%	24

† *Full nodal response (pathologically cancer free),* ‡*Partial nodal response (pN < cN),* §*Nodal progression (pN > cN)*

Table 3b. cN compared to pN after 46 Gy of IMRT using 8th TNM classification

p16 positive OPSCC – 8th TNM classification

Clinical nodal stage	*Pathological nodal stage (after neckdissection)*						
	pN0		**pN1**		**pN2**		**Total**
	N	*%*	*N*	*%*	*N*	*%*	
cN1	20†		27		0		47
cN2	3†		1‡		1		5
cN3	0		1‡		0		1
Total	23	43.4%	29	54.7%	1	1.9%	53

† *Full nodal response (pathologically cancer free),* ‡*Partial nodal response (pN < cN)*

p16 negative OPSCC – 8th TNM classification

Clinical nodal stage	*Pathological nodal stage (after neckdissection)*												
	pN0		**pN1**		**pN2a**		**pN2b**		**pN3a**		**pN3b**		**Total**
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*
cN1	5†		5		0		3§		0		0		13
cN2a	0		0		2		0		0		0		2
cN2b	0		1‡		0		4		0		0		5
cN3a	0		0		0		1‡		0		0		1
cN3b	0		0		0		0		0		3		3
Total	5	20.8%	6	25.0%	2	8.3%	8	33.3%	0	0%	3	12.5%	24

† *Full nodal response (pathologically cancer free),* ‡*Partial nodal response (pN < cN),* §*Nodal progression (pN > cN)*

Using the 7th TNM classification, nodal response (partial or complete) was significantly associated with HPV status (p=0.002). 67.9% (n=36) of p16-positive patients had complete or partial response compared to 29.2% (n=7) of p16-negative patients (Supplementary Table A1). Multivariate logistic regression analysis was performed to estimate the adjusted Odds Ratio (OR) of the effect of HPV status on nodal response, adjusted for UICC/AJCC tumor stage and tobacco use. p16-positive patients had an OR of 4.6 (95% CI 1.4 – 15.5, p=0.012) to achieve complete nodal response after 46 Gy of IMRT. However, smoking interacts with this effect. Patients with p16-positive OPSCC who were non- or former smokers had an OR of 6.3 (95% CI 1.4 – 28.4, p=0.017), whereas p16-positive current smokers had an OR of 4.5 (95% CI 0.7 – 25.7, p=0.089) to achieve complete nodal response.

When applying the 8th TNM classification, complete or partial response seemed to be related to HPV status, however not significantly (p=0.138) (Supplementary Table A2). Of the p16-positive patients, 47.2% (n=25) compared to 29.2% (n=7) of the p16-negative patients had nodal response. The same association was seen in multivariate analysis, adjusted for UICC/AJCC tumor stage and tobacco use, with an OR of 1.7 (p=0.314). Using the 8th TNM classification, the correlation between nodal response and p16 positivity does not hold.

Effect of the Rotterdam protocol, HPV status and nodal response on overall and disease free survival

Mean 5-year OS was 77.8%. The log rank test for OS significantly favored patients with p16-positive OPSCC (5-year OS 87.5%) versus p16-negative OPSCC (5-year OS 55.8%, p<0.001). The same result was found for 5-year DFS of patients with p16-positive OPSCC (98%) versus 54.5% DFS for p16-negative OPSCC (p<0.001), see Figure 2a-b. When HPV status was stratified for smoking status OS was significantly better in never or former smokers (5-year OS p16-positive disease 95% versus 71.6% p16-negative disease), then in current smokers (5-year OS p16-positive disease 62.5%, p16-negative disease 41.7%, p<0.001).

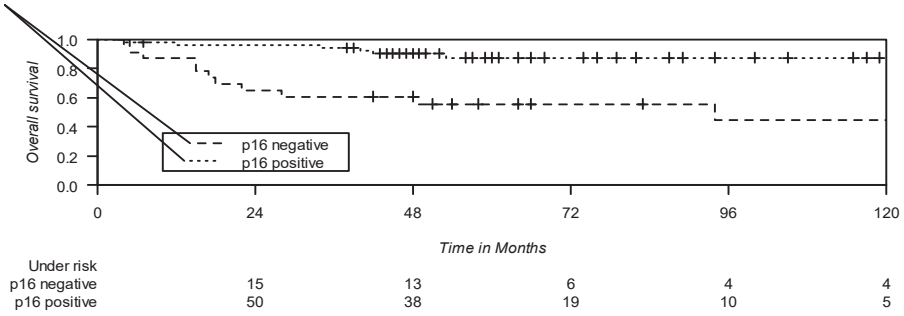
Patients with complete nodal response after 46 Gy of IMRT had a 5-year OS of 96.3% versus 67.0% for patients with partial or no nodal response (p= 0.003).

5-year OS for p16-positive patients with complete nodal response was 100%, and 80% for p16-negative patients. 5-year OS for p16-positive patients with partial or no nodal response was 78.5% and for p16-negative patients 51.5%. Both are significant results (p< 0.001). Similar trends were seen for DFS, but these findings did not reach statistical significance.

Figure 3 shows the difference between 7th and 8th TNM classification in classifying p16-positive OPSCC patients in prognostic subgroups with regards to nodal response.

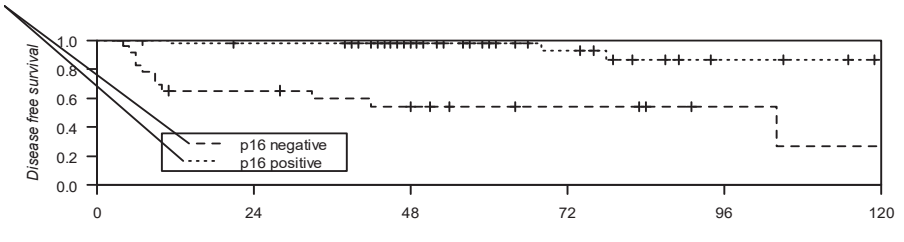
Figure 2. Kaplan Meier curve of overall (2a) and disease free survival (2b) as a function of p16 immunostaining

2a.



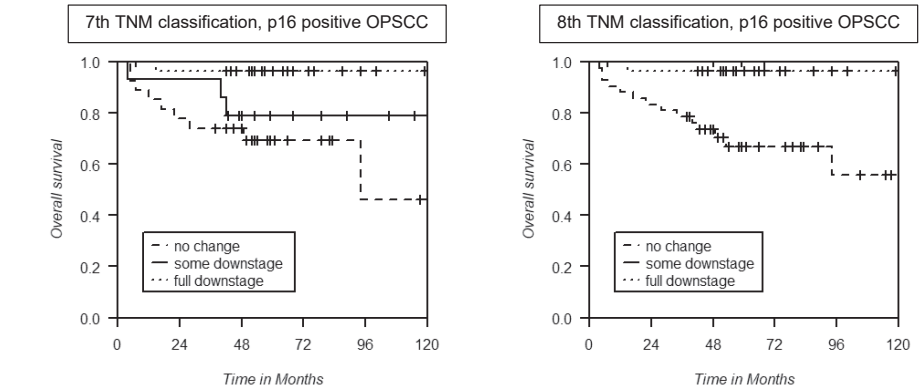
Under risk					
p16 negative	15	13	6	4	4
p16 positive	50	38	19	10	5

2b.



Under risk					
p16 negative	13	9	5	2	1
p16 positive	49	37	17	7	4

Figure 3. Kaplan Meier curve of overall survival as a function of p16 immunostaining and nodal response according to 7th and 8th TNM classification respectively



Under risk					
no change	21	17	6	2	1
some downstage	13	9	5	3	1
full downstage	26	22	13	9	7

Under risk					
no change	35	25	12	5	2
some downstage	3	3	0	0	0
full downstage	26	22	13	9	7

The Kaplan Meier curves illustrate that the use of the 8th TNM classification provides a more realistic effect of nodal response on cumulative survival for patients with p16-positive OPSCC compared to the 7th edition. By classifying nodal stage (cN and pN) according to the 8th edition, both partial and complete nodal response lead to a 100% survival, even after 10 years. This is in contrast to the 7th edition where overall survival of p16-positive patients with partial nodal response is over 90%.

DISCUSSION

In this study we observed that the majority of our patients with T1-2 node positive OPSCC had p16-positive disease. Treatment with the Rotterdam protocol for OPSCC resulted in excellent loco regional control and overall survival was significantly better in this group compared to patients with p16-negative OPSCC.

This current study shows that p16-positive disease is associated with increased nodal response after a relatively low dose of IMRT to the involved neck. Patients with complete nodal response after IMRT had a significantly increased survival compared to patients with partial nodal response. However, this association is affected by the TNM classification that is used. In the 8th TNM classification, stage grouping for T1-2 OPSCC is based entirely on the extent of nodal disease, and permits a more appropriate depiction of the prognosis of HPV positive disease.[7] Our hypothesis of the favorable effect of HPV positive disease on nodal response after 46 Gy of IMRT holds true using the 7th TNM classification but is obscured by the use of the 8th TNM classification. This finding is not that remarkable since advanced nodal disease for p16-positive OPSCC is literally down-staged in the 8th TNM classification, for both cN and pN.[7,8] However, this finding does point out that the 8th TNM provides a more realistic effect of nodal response on overall survival for p16-positive OPSCC patients. Classification of nodal stage by the 8th edition shows a 100% overall survival for p16-positive patients with both partial and complete nodal response in contrast with over 90% overall survival for patients with partial nodal response that are staged using the 7th edition.

Yet, our results also show a proportion of p16-positive OPSCC patients with no nodal response after 46 Gy of IMRT. These patients also have a reduced 5 year overall survival (75.7%). There are two possible explanations for these results: false positivity of p16 analysis and smoking. Since we did not carry out HPV DNA-detection, some p16-positive tumors may have been HPV negative, with relatively worse prognosis. Furthermore, not all FFPE's were available of all patients treated according to the Rotterdam protocol between 2000 and 2012, and therefore selection bias might have occurred. In addition, in our study

we also found that smoking negatively interacts with the effect of p16-positive OPSCC on prognosis. This finding is in conjunction with the study by Platek et al., where smoking was found to be a prognostic factor independent of HPV.[24] They show that current smoking during radiotherapy in OPSCC patients is associated with a four- to sevenfold increase in risk of mortality for HPV positive and HPV negative patients respectively. Our study confirms that every effort should be made to motivate current smokers with OPSCC to stop smoking.

Our results on p16-positive disease being significantly associated with increased nodal response even after 46 Gy radiotherapy are in line with those found in literature. Bird et al., showed that only 9% of HPV-positive patients underwent neck dissection within 6 months of radiation completion (54-65 Gy of IMRT) because of suspected residual disease.[25] In addition, Garden et al. found that 80% of HPV-positive patients had no neck dissection based on their response after 70 Gy of IMRT.[26] They concluded that only 2% of HPV-positive OPSCC patients benefit from a neck dissection.

However, Marklund et al. found that HPV-positive tumors had the same proportion (23%) of viable tumor cells in the neck specimen, 6-8 weeks after radiotherapy (64-68 Gy) as HPV-negative tumors.[10] In our study, neck dissection was performed within 3 weeks after radiation. An explanation for the difference between these results could be that HPV-positive tumors have a more rapid early response after radiotherapy followed by tapered response such that it may take longer for HPV-positive nodes to regress. Other studies performed weekly CT scans to measure the volume of positive lymph nodes.[27,28] They found that in HPV-positive patients with node positive OPSCC, spontaneous shrinkage was seen before radiotherapy, during treatment enlargement of nodes was seen and shortly after treatment there was a poor response on IMRT (25.3% failed to show complete response after 12 weeks). This finding suggests that complementary neck dissection still seems necessary for a subgroup of these patients. Our data also support this suggestion following the decreased overall- and disease free survival in patients who had no nodal response 3 weeks after 46 Gy of IMRT.

CONCLUSION

The Rotterdam protocol for T1-2 OPSCC results in excellent local control rates with low toxicity. In node-positive disease, neck-dissection after a relatively low dose of IMRT to the involved neck results in excellent regional control and a low severe complication rate. p16-positivity is associated with complete nodal response. Smoking, however, negatively interacts with this effect. There is a significant difference between HPV-positive tumors

with complete nodal response (pN0) and HPV-negative tumors in terms of survival. Despite these results, we believe there is not enough evidence to omit neck dissection yet. On the one hand due to the relatively large fraction of p16-positive patients in this study that did not show nodal response. On the other hand due to the fact that p16-positivity does not equal HPV positivity and p16-positive/HPV DNA negative OPSCC do not have the HPV-related favorable prognosis.[29] Therefore, additional HPV DNA testing should be considered when looking at decisions regarding treatment deintensification. Nevertheless, the results of this and other studies suggest that a number of OPSCC patients do not necessarily need the current gold standard of 70 Gy of radiotherapy to obtain locoregional control of their disease. Currently proceeding treatment de-intensification trials may provide a proof of this principle.[14-16] In addition, our center is embarking on a prospective study using functional MRI to assess the neck prior to neck dissection for OPSCC patients.

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Supplementary Table A1. Univariate analysis of associations between variables and nodal response, based on 7th TNM classification

		Complete or partial nodal response						
		No			yes			
		Mean	N	%	Mean	N	%	p-value*
Age		60			57			0.304
Gender	Male		23	67.6%		34	79.1%	0.256
	Female		11	32.4%		9	20.9%	
ACE27	None		14	41.2%		16	37.2%	0.609
	Mild		8	23.5%		16	37.2%	
	Moderate		10	29.4%		9	20.9%	
	Severe		2	5.9%		2	4.7%	
Tobacco use	No smoking history		4	11.8%		12	27.9%	0.149
	Former smoker		16	47.1%		20	46.5%	
	Current smoker		14	41.2%		11	25.6%	
Clinical tumor stage (7th UICC/AJCC classification system)	I		0	0.0%		0	0.0%	0.182
	II		0	0.0%		0	0.0%	
	III		15	44.1%		12	27.9%	
	Iva		19	55.9%		29	67.4%	
	IVb		0	0.0%		2	4.7%	
p16 immunostaining	Negative		17	50.0%		7	16.3%	**0.002**
	Positive (>70% staining)		17	50.0%		36	83.7%	
Acute toxicity	Low (0-2)		28	82.4%		38	88.4%	0.454
	High (3-4)		6	17.6%		5	11.6%	
Recurrent disease	No recurrent disease		25	73.5%		41	95.3%	**0.025**
	(Loco)regional recurrence		4	11.8%		1	2.3%	
	Distant metastasis		5	14.7%		1	2.3%	
Deceased	No		20	58.8%		38	88.4%	**0.003**
	Yes		14	41.2%		5	11.6%	

*univariate analysis by chi-square test independent samples t-test, $p < 0.05$ is considered significant result