Published in final edited form as:

Head Neck. 2016 February ; 38(2): 184–190. doi:10.1002/hed.23882.

Matted Nodes Predict Distant Metastasis in Advanced Stage III/IV Oropharyngeal Squamous Cell Carcinoma

Matthew E Spector, MD¹, Steven B Chinn, MD¹, Emily Bellile, MS², K Kelly Gallagher, MD¹, Mohannad Ibrahim, MD³, Jeffrey Vainshtein, MD⁴, Eric J Chanowski, MD¹, Heather M Walline, MS¹, Jeffrey S Moyer, MD¹, Mark E Prince, MD¹, Gregory T Wolf, MD¹, Carol R Bradford, MD¹, Jonathan B McHugh, MD⁵, Thomas Carey, PhD¹, Francis P Worden, MD⁶, Avraham Eisbruch, MD⁴, and Douglas B Chepeha, MD, MSPH¹

¹Department of Otolaryngology – Head and Neck Surgery, University of Michigan Health System, Ann Arbor, MI, USA

²Department of Biostatistics, University of Michigan Health System, Ann Arbor, MI, USA

³Department of Radiology, University of Michigan Health System, Ann Arbor, MI, USA

⁴Department of Radiation Oncology, University of Michigan Health System, Ann Arbor, MI, USA

⁵Department of Pathology, University of Michigan Health System, Ann Arbor, MI, USA

⁶Department of Medical Oncology, University of Michigan Health System, Ann Arbor, MI, USA

Abstract

Background—We recently described the imaging characteristics of multiple confluent regional metastases (matted nodes) and found that this characteristic was associated with distant metastasis in patients with oropharyngeal squamous cell carcinoma (OPSCC). The purpose of this study is to determine if matted nodes are a predictive marker for distant metastasis.

Methods—Radiologic lymph node characteristics on 205 untreated stage III/IV with OPSCC patients of whom 192 had known HPV status underwent weekly carboplatin and paclitaxel with concomitant IMRT between 2003–2010 with minimum 2 years of follow-up.

Results—The 3-year DSS for patients with matted nodes was 58% versus 97% with non-matted nodes ($p=0.0001$). The prevalence of matted nodes in the population was 20%. The positive predictive value of matted nodes for distant metastasis is 66%, and the negative predictive value is 99%.

Conclusions—Matted nodes are a predictive marker for distant disease and can be used for planning new clinical interventions.

Keywords

oropharyngeal squamous cell carcinoma; matted nodes; predictor; prognosis; biomarkers; distant metastasis

Corresponding Author: Douglas B Chepeha MD, MSPH, 1904 Taubman Center, 1500 E Medical Center Drive, Ann Arbor, MI 48109, Phone: (734) 936-3172, Fax: (734) 647-9691, dchepeha@med.umich.edu.

Conflicts of interest and financial disclosures: None

Introduction

Treatment failure from distant metastasis in patients with oropharyngeal squamous cell carcinoma (OPSCC) has doubled over the past 20 years and now accounts for approximately 45% of deaths.^{1, 2} Several factors account for the increased prevalence of distant metastasis as the site of first failure in OPSCC. First, excellent locoregional control rates have shifted the pattern of failure toward distant metastasis.² Second, the proportion of patients who die of other causes is lower because the cohort presenting with OPSCC are younger, healthier and have a lower incidence of tobacco use. This decreases the deaths from other causes and increases the relative proportion of patients who may go on to develop distant metastasis. In addition, human papillomavirus-associated tumors (HPV) may have altered the patterns of relapse with an increased rate of distant metastasis.³⁻⁵

With the current interest in de-escalating treatment of patients with OPSCC, it would be beneficial to identify those patients at risk for distant disease so that they could be excluded from de-escalation trials and alternative treatments could be evaluated. There has been previous work that has evaluated prognostic markers of distant metastasis. For example, preferential expression of Micro-RNAs⁶, combination advanced T and N classification⁷, and active smoking status⁸ have all been evaluated as prognostic markers. The ideal way to identify patients at risk for distant metastasis would be *predictive* in nature, so that patients could be identified in a pretreatment fashion to help develop interventions to prevent treatment failure. Our previous work has identified matted nodes (defined as three nodes abutting one another with loss of intervening fat plane that is replaced with radiologic evidence of extracapsular spread) as a *prognostic* marker for patients with OPSCC, but the *predictive* value is unknown.⁵ The purpose of this study is to define the predictive value of matted nodes for distant metastasis and survival in patients with OPSCC.

Methods

Study Population

All patients were treated under a uniform clinical protocol consisting of weekly concomitant carboplatin, paclitaxel and intensity modulated radiation therapy (IMRT) for advanced stage (III,IV) OPSCC between 2003 and 2010. Staging was performed in accordance with the 2010 American Joint Committee on Cancer staging system with clinical exam, direct laryngoscopy in the operating room and computed tomography (CT) scan and/or computed tomography/positron emission tomography scan (CT/PET). Patients were excluded if they had previous surgery or radiation therapy to the upper aerodigestive tract or if neck imaging was not performed within 4 weeks of the initiation of treatment.

Population characteristics

There were 215 patients who were treated with this regimen. Ten patients were excluded from this study because pretreatment imaging was unavailable for review or they had excisional lymph node biopsies prior to referral and definitive CT scan. Two-hundred five previously untreated patients were identified and baseline characteristics are shown in Table 1. There were 183 male patients and the mean age of the cohort was 56.3 years. The

frequencies of involved subsites were 43% (89/205) base of tongue, 53% (109/205) tonsil, 1% (3/205) glossotonsillar sulcus, 1% (3/205) posterior pharyngeal wall, and 1% (1/205) soft palate. There were 33% (67/205) who had T4 tumors and 89% (183/205) who were stage IV. Tobacco status was defined categorically as never, prior [quit greater than 6 months prior to diagnosis], or current use of cigarettes, cigars, pipe, chewing tobacco, snuff or snus. There were 61 never tobacco users, 72 prior tobacco users, and 72 current tobacco users.

Treatment protocol

Radiation therapy was delivered using daily fractionated IMRT, 5 days per week over 35 fractions. Prescription doses were 70 Gy at 2.0 Gy per fraction to gross disease and 59–63 Gy at 1.7–1.8 Gy per fraction to low- and high-risk subclinical regions, respectively, delivered concomitantly according to published methods.^{9, 10} Chemotherapy consisted of weekly carboplatin (AUC 1) intravenous over 30 minutes and paclitaxel (30 mg/m²) intravenous over 1 hour. Hydration and antiemetics were administered according to the standard of care.

Tissue microarray and immunostaining

A tissue microarray (TMA) was constructed from pretreatment biopsies of the primary tumor for 176 of the 205 patients. Of the remaining patients, 10 had sufficient tissue to obtain a single slide from the paraffin embedded biopsy and 19 did not have adequate tissue samples available for analysis. The TMA and single slides were processed by a previously described method.¹¹ Staining for p16 was performed per protocol supplied by the kit (CINtec p16INK4a Histology Kit; mtm Laboratories, Westborough MA). Antibody binding was scored by a pathologist (JBM), using a continuous scale (ie, 10%, 30%, 90%, etc.) for the proportion of p16-positive tumor cells in each core or slide and percentage scored was broken down into an ordinal scale of 1 to 4: 1 was less than 5%; 2, 5% to 20%; 3, 21% to 50%; and 4, 51% to 100% tumor staining. Intensity was scored as 1 equal to no staining; 2, low intensity; 3, moderate; and 4, high intensity. Scores for multiple cores from each patient were averaged. Staining for p16 was considered positive when >75% of tumor cells demonstrated strong nuclear and cytoplasmic staining or >2 intensity.

Isolation of DNA from separately cored tissue samples was performed using the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA). DNA concentration and purity were confirmed via NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA). HPV status was determined by an ultra-sensitive method using real-time competitive polymerase chain reaction and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy with separation of products on a matrix-loaded silicon chip array.¹²

HPV status was considered positive if *either* p16 staining was positive or PCR assay was positive. Table 2 shows the discordances between p16 staining and PCR assay results. The HPV status was known for 186 out of 205 patients and was positive in 92% of cases. The pathologist was blinded to the clinical outcome.

Pretreatment imaging

Pretreatment CT or CT/PET scans within four weeks of starting therapy were reviewed by a neuroradiologist (MI). Primary tumor site and size, distance of the primary tumor from the midline, and encasement of the carotid artery by the primary tumor were recorded. The size (largest two dimensions) and distribution (level I–V) of each lymph node was recorded for each level of the neck. AJCC N3 disease was defined clinically as a lymph node or group of lymph nodes greater than six centimeters. *Matted nodes* were defined as three nodes abutting one another with loss of intervening fat plane that is replaced with radiologic evidence of extracapsular spread (Figure 1). Extracapsular spread (ECS) was defined with imaging as loss of the sharp plane between the capsule of the lymph node and the surrounding fat.

Statistical analysis

Outcomes of interest were overall survival [OS], disease-specific survival [DSS], and pattern of recurrence (local, regional, distant). Survival estimates were defined from date of diagnosis and were computed using the Kaplan-Meier method. Bivariate associations between matted nodes and other clinical variables were tested with exact tests (i.e. Fisher's exact test, chi-square test with Monte Carlo estimates for error terms). The log-rank test was used to evaluate univariate associations of survival and Cox models were used to evaluate the multivariate association of matted nodes with survival after controlling for clinical variables including age, T classification, tobacco status, and HPV status. This research was approved by Institutional Review Board for human experimentation of the University of Michigan.

Results

The objective of this study was to ascertain the predictive value of matted nodes. The prevalence of matted nodes in the study population was 20%. There were 42 patients identified with matted nodes, with 28 (67%) of these patients developing distant metastasis. Of the 30 patients in the cohort that developed distant metastasis, 28 (93%) had matted nodes. The positive predictive value of developing distant metastasis when matted nodes are present is 66%, and the negative predictive value is 99%. The sensitivity and specificity of matted nodes to predict distant metastasis was 93 and 92%, respectively. The 3-year overall survival was 83% and disease specific survival was 88% for the cohort with a median follow-up 43 months.

The 3-year DSS for patients with matted nodes was 58% and for patients with no evidence of matted nodes was 97% (Figure 2, $p=0.0001$).

There were 186 patients who had HPV status determined and available for analysis. Of the 186 patients, there were 171 who were HPV positive. With univariate analyses, HPV positive patients experienced a significantly improved DSS (3yr: 88% vs 69%, $p=0.05$) and OS (3yr: 86% vs 60%, $p=0.02$) when compared to HPV negative patients (Figure 3). There was no significant correlation of HPV status to the finding of matted nodes ($p=0.1$) (this is a strong trend however, particularly with such a small number of HPV- patients). Although

there are only 15 HPV negative patients, this suggests that the predictive value of matted nodes is independent of HPV status. With univariate analyses, there was no significant difference in disease specific survival when stratifying by tobacco use (Figure 4, $p=0.69$). There were 61 never tobacco users, 72 prior tobacco users, and 72 current tobacco users. The 3-year DSS was 90%, 85%, and 88% in the never, prior, and current tobacco use groups, respectively. The correlation between matted nodes to tobacco status was not statistically significant ($p>0.2$).

The prevalence of radiologic ECS in our cohort was 63% (129/205), consistent with our previous work. Patients with matted nodes, as we have previously defined, have ECS. 42.4% (87/205) of the entire cohort had ECS without evidence of matted nodes. Figure 5 shows the overall and disease specific survival of patients without ECS (blue curve), ECS with non-matted nodes (green curve), and ECS with matted nodes (tan curve). There is no difference in overall or disease specific survival in patients without ECS and patients with ECS and non-matted nodes.

Bivariate testing showed that matted nodes were predictive of survival within the AJCC nodal classification system. There were 79 patients who presented with AJCC N2b disease. Of these 79 patients, 16 patients had matted nodes and 63 had no evidence of matted nodes. There was a significant difference in the DSS in patients with N2b disease when stratifying by matted nodes (Figure 6a, $p=0.0001$). There were 51 patients who presented with AJCC N2c disease. Of these 51 patients, 12 patients had matted nodes and 39 had no evidence of matted nodes. There was a significant difference in the DSS in patients with N2c disease when stratifying by matted nodes (Figure 6b, $p=0.0001$). There were 29 patients who presented with AJCC N3 disease. Of these 29 patients, 14 patients had matted nodes and 15 had no evidence of matted nodes. There was a significant difference in the DSS in patients with N3 disease when stratifying by matted nodes (Figure 6c, $p=0.009$). Matted nodes independently predicted survival within each AJCC nodal classification.

A bivariate analysis of the prognostic value of matted nodes was evaluated within the following variables: HPV status, tobacco use, and T classification. Matted nodes were a poor prognostic factor of disease specific survival in HPV positive patients ($p=0.0001$) but not in HPV negative patients ($p=0.14$). The HPV negative group contained 15 patients and was underpowered. Matted nodes were a poor prognostic factor among never ($p=0.0001$), ever ($p=0.0001$) and current ($p=0.0001$) tobacco users. In addition, matted nodes were a poor prognostic factor in patients with low T classification (T1/T2, $p=0.0001$) and high T classification (T3/T4, $p=0.0001$).

Multivariate analysis was performed using matted nodes, age, T classification, HPV status, and smoking status and is shown in Table 3. Matted nodes were an independent predictor of poor prognosis when controlling for these variables (DSS hazard ratio 14.1; 95% confidence interval, 6.0 – 33.1; $p<0.0001$)

Discussion

This study shows that matted nodes are an important independent predictor of distant metastasis and survival in patients with oropharyngeal carcinoma. The predictive ability of matted nodes facilitates the identification of patients who might benefit from novel treatment interventions to prevent the development or progression of distant metastasis. The absence of matted nodes also helps identify patients who are at lower risk for distant treatment failure.

Matted nodes are not a surrogate marker for extensive neck disease or an alternative description of massive neck involvement for nodal metastasis. The analysis presented in this paper shows that matted nodes are present in patients with N2b, N2c, and N3 disease, and within these AJCC classifications, are associated with higher rates of distant metastasis (Figure 6). Previous work has stratified patients with nodal metastasis into risk groups. Ang and colleagues categorized patients with N2b or N2c disease and greater than 10 pack years of smoking into an intermediate risk category for recurrence.¹ O'Sullivan et al. found that patients treated with chemoradiation who had T4 or N3 disease had higher rates of distant disease.⁷ We believe that the characteristics associated with matted nodes may extend these findings and will be a more specific marker for distant metastasis. It is particularly important to note that the imaging characteristics that reflect the biology of metastases to multiple regional lymph nodes are independent of overall tumor stage, smoking history and perhaps even HPV status. Better understanding of the molecular characteristics associated with the imaging finding of matted nodes versus non-matted nodes is likely to reveal factors directly associated with metastatic behavior and potentially new targets for therapy of disseminated disease.

There are several potential weaknesses in this study. The predictive value of matted nodes needs to be validated in more than one institution and in more than one protocol. In addition, the analysis is "post hoc" and to be sure of the predictive value of matted nodes, it would have to be tested in a prospective study. Also our treatment protocol is not the most commonly used regimen of concurrent chemotherapy and radiation. Patients were treated with carboplatin and paclitaxel, which could be inferior to high dose cisplatin in patients with OPSCC. Having stated this, the overall survival and disease specific survival in our cohort compares favorably to high dose cisplatin regimens.¹³

Combined treatment of OPSCC has effectively improved control of local and regional disease.^{2, 7, 9} The subset of patients who develop distant metastatic disease do so without a clear biologic marker. Matted nodes are a clinically useful predictor of distant metastasis in OPSCC that is associated with poor survival. For patients who are at risk for developing distant disease, matted nodes appear to be a useful clinical predictor of this aggressive phenotype. We lack pathological information about matted nodes because it is a radiologic finding and no pathologic correlate was available because all patients were managed nonsurgically. Whether matted nodes represent extensive ECS or an immunologic response is unknown, but our findings certainly suggest that matted nodes represent a marker of a biologically different disease.

The positive predictive value of developing distant metastasis when matted nodes are present is 66%, and the negative predictive value is 99%. The sensitivity and specificity of matted nodes to predict distant metastasis is 93 and 92%, respectively. The predictive ability of matted nodes facilitates the identification of patients who may benefit from novel treatment interventions to prevent the development or progression of distant metastasis.

Acknowledgments

Financial support: P50 CA97248 NIH NCI NIDCR SPORE

References

1. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010; 363:24–35. [PubMed: 20530316]
2. Daly ME, Le QT, Maxim PG, et al. Intensity-modulated radiotherapy in the treatment of oropharyngeal cancer: clinical outcomes and patterns of failure. *Int J Radiat Oncol Biol Phys*. 2010; 76:1339–1346. [PubMed: 19540068]
3. Müller S, Khuri FR, Kono SA, Beitler JJ, Shin DM, Saba NF. HPV positive squamous cell carcinoma of the oropharynx. Are we observing an unusual pattern of metastases? *Head Neck Pathol*. 2012 Sep; 6(3):336–44. Epub 2012 Apr 15. 10.1007/s12105-012-0355-6 [PubMed: 22528827]
4. Huang SH, Perez-Ordóñez B, Weinreb I, et al. Natural course of distant metastases following radiotherapy or chemoradiotherapy in HPV-related oropharyngeal cancer. *Oral Oncol*. 2013 Jan; 49(1):79–85. Epub 2012 Aug 20. 10.1016/j.oraloncology.2012.07.015 [PubMed: 22917550]
5. Spector ME, Gallagher KK, Light E, et al. Matted nodes: poor prognostic marker in oropharyngeal squamous cell carcinoma independent of HPV and EGFR status. *Head Neck*. 2012 Dec; 34(12):1727–33. Epub 2012 Jan 13. 10.1002/hed.21997 [PubMed: 22247002]
6. Hui AB, Lin A, Xu W, Waldron L, et al. Potentially Prognostic Micro-RNAs in HPV-Associated Oropharyngeal Carcinoma. *Clin Cancer Res*. 2013 Mar 4. Epub ahead of print.
7. O'Sullivan B, Huang SH, Siu LL, et al. Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. *J Clin Oncol*. 2013 Feb 10; 31(5):543–50. Epub 2013 Jan 7. 10.1200/JCO.2012.44.0164 [PubMed: 23295795]
8. McBride SM, Ali NN, Margalit DN, Chan AW. Active tobacco smoking and distant metastasis in patients with oropharyngeal cancer. *Int J Radiat Oncol Biol Phys*. 2012 Sep 1; 84(1):183–8. Epub 2012 Feb 11. 10.1016/j.ijrobp.2011.11.044 [PubMed: 22330993]
9. Feng FY, Kim HM, Lyden TH, et al. Intensity-modulated chemoradiotherapy aiming to reduce dysphagia in patients with oropharyngeal cancer: clinical and functional results. *J Clin Oncol*. 2010 Jun 1; 28(16):2732–8. Epub 2010 Apr 26. [PubMed: 20421546]
10. Feng FY, Kim HM, Lyden TH, et al. Intensity-modulated radiotherapy of head and neck cancer aiming to reduce dysphagia: early dose-effect relationships for the swallowing structures. *Int J Radiat Oncol Biol Phys*. 2007 Aug 1; 68(5):1289–98. Epub 2007 Jun 7. [PubMed: 17560051]
11. Kumar B, Cordell KG, Lee JS, et al. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *J Clin Oncol*. 2008 Jul 1; 26(19):3128–37. Epub 2008 May 12. [PubMed: 18474878]
12. Tang AL, Hauff SJ, Owen JH, et al. UM-SCC-104: a new human papillomavirus-16-positive cancer stem cell-containing head and neck squamous cell carcinoma cell line. *Head Neck*. 2012 Oct; 34(10):1480–91. [PubMed: 22162267]
13. Dobrosotskaya IY, Bellile E, Spector ME, et al. Weekly chemotherapy with radiation versus high-dose cisplatin with radiation as organ preservation for patients with HPV positive and HPV negative locally advanced squamous cell carcinoma of the oropharynx (SCCOP). *Head Neck*. 2013 Apr 18. 10.1002/hed.23339

Figure 1. Three dimensional reconstruction of a patient with matted nodes. There are four nodes shown in orange, green, yellow and purple shown with loss of intervening fat plane lateral to the jugular vein (blue) and carotid artery (red).

Figure 2.

Kaplan–Meier survival curves of the entire cohort stratified by the presence of matted nodes. The 3-year DSS for patients with matted nodes was 58% and for patients with no evidence of matted nodes was 97% ($p=0.0001$).

Figure 3.

Kaplan–Meier survival curves of the entire cohort stratified by Human Papilloma Virus (HPV) status. The 3-year DSS for HPV positive patients was 88% and for HPV negative patients was 69% ($p=0.05$).

Figure 4. Kaplan–Meier survival curves of the entire cohort stratified by smoking status. The 3-year DSS was 90%, 85%, and 88% in the never, prior, and current tobacco use groups, respectively. There were no significant differences between the groups.

Figure 5.

Kaplan–Meier survival curves of the entire cohort stratified by ECS. Patients were categorized as having no ECS (blue curve), ECS with non-matted nodes (green curve), and ECS with matted nodes (tan curve).

A There was no difference in OS in patients without ECS and patients with ECS and non-matted nodes. ($p=0.67$).

B There was no difference in DSS in patients without ECS and patients with ECS and non-matted nodes. ($p=0.48$).

Figure 6.

A. Kaplan–Meier survival curves of patients with N2b nodal disease stratified by matted nodes. There was a significant difference in the DSS in patients with N2b disease when stratifying by matted nodes ($p=0.0001$).

B. Kaplan–Meier survival curves of patients with N2c nodal disease stratified by matted nodes. There was a significant difference in the DSS in patients with N2c disease when stratifying by matted nodes ($p=0.0001$).

C. Kaplan–Meier survival curves of patients with N3 nodal disease stratified by matted nodes. There was a significant difference in the DSS in patients with N3 disease when stratifying by matted nodes ($p=0.009$).

Table 1

Baseline Characteristics of the Entire Cohort

Characteristics		Entire Cohort n=205
Age	mean	56.3
Sub-Site	BOT	43% (89)
	Tonsil	53% (109)
	Other Sub-Site	4% (7)
Overall Stage	III	11% (22)
	IV	89% (183)
T stage	T1	17% (36)
	T2	32% (65)
	T3	18% (37)
	T4	33% (67)
N classification	N0	7% (14)
	N1	7% (15)
	N2a	8% (17)
	N2b	39% (79)
	N2c	25% (51)
	N3	14% (29)
HPV Status	Positive	84% (171)
	Negative	7% (15)
	Missing	9% (19)
Tobacco Status	Never	30% (61)
	Prior	35% (72)
	Current	35% (72)

Table 2

Patients with Discordances between p16 Staining and PCR Assay Results

Patient	PCR Assay Results	p16 Immunostain Proportion	HPV Status for Analysis
1	Negative	4	Positive
2	Negative	4	Positive
3	HPV16	0	Positive
4	Negative	4	Positive
5	HPV16	0	Positive

p16 Immunostain Proportions: 1= <5%, 2=5–20%, 3=21–50%, 4=51–100% Patients are considered p16 positive if there is >5% staining pattern present

Table 3

Multivariable Cox Model for Disease Specific Survival, n=205

Parameter	Hazard Ratio	95% Hazard Ratio Confidence Limits		p-value
Matted Nodes	14.102	6.002	33.131	<.0001
Age	1.035	0.986	1.086	0.1700
T stage (T1/T2 vs T3/T4)	1.926	0.804	4.614	0.1417
HPV Status	1.118	0.407	3.068	0.8291
Current Smoker (vs. Never)	0.888	0.356	2.213	0.7983
Ever Smoker (vs. Never)	0.853	0.335	2.169	0.7383