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HEAD AND NECK

The clinical impact of p16 status in fine-needle aspirates of cervical lymph node metastasis of head and neck squamous cell carcinomas

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Abstract Lymph node involvement is prognostically the most determinant clinical factor for patients with head and neck squamous cell carcinomas (HNSCCs). Ultrasound of the neck and fine-needle aspiration (FNA) cytology is one of the first diagnostic procedures and the most accurate diagnostic staging tool for the neck. Patients with HPV-positive oropharyngeal carcinomas (OPSCC) show a significantly better prognosis when compared with HPV-negative OPSCC. P16 overexpression is accepted as surrogate marker for HPV-positive in OPSCC. These HPV/p16-positive OPSCC are localized either in the palatal tonsils or the base of tongue and frequently present with lymph node metastases. We analyzed the correlation and reliability of p16 expression of the FNA of the lymph node metastasis with the immunohistochemical expression of p16 of the same lymph node metastasis and its corresponding primary tumor, as it could be of importance for determining the localization and different prognosis of the primary tumor. 54 HNSCC patients were evaluated, p16 expression of the primary tumors and their lymph node metastases correlated precisely. In 25 of the 54 HNSCC patients, a FNA of the lymph node metastases was taken before the treatment. The positive cytological and immunohistochemical p16 staining correlated exactly. Of the 17 histologically p16-negative lymph node metastases 15 FNA were p16-negative, whereas two samples were p16-positive. In our view, a cytological p16 analysis of cervical lymph node metastasis can facilitate the correct localization of the primary tumor and discriminate reliably HPV-positive OPSCC from HPV-negative HNSCC with their significantly diverse prognosis.

Keywords Head and neck cancer · HPV · p16 · Fine-needle aspiration · Oropharyngeal cancer

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Introduction

Patients with head and neck squamous cell carcinoma (HNSCC) frequently present with cervical lymph node metastases. Lymph node involvement is the determinant clinical prognostic factor for patients with HNSCC. The 5-year-survival rate of lymph node positive, advanced-stage HNSCC falls below 50 %. Ultrasound (US) of the neck and fine-needle aspiration (FNA) cytology of the suspicious lymph nodes is one of the first diagnostic procedures in a clinical examination for a thorough work up of the neck. US and FNA represent the most accurate diagnostic tools for a precise neck staging. These methods are easily available, quickly performed, safe, and cost effective. The hit rate for a correct diagnosis is high and can surpass the rate of conventional radiologic techniques such as computer tomography (CT) or magnetic resonance imaging (MRI) [1].

Cancer statistics report an increasing incidence of oropharyngeal squamous cell carcinoma (OPSCC) [2–4]. This subgroup of HPV 16- or 18-positive OPSCC is characterized by distinct molecular characteristics such as

an over expression of p16 suggested to be specific for HPV-positive OPSCC [5, 6]. Patients with HPV-positive or p16-positive OPSCC show a significantly improved prognosis when compared with patients with HPV- or p16-negative OPSCC [7]. Compared with negative HNSCC, these HPV/p16-positive OPSCC frequently present with lymph node metastases [8, 9]. HPV/p16-positive OPSCC are mainly localized in the palatal tonsils or at the base of the tongue [10]. These tumors can sometimes only be detected by panendoscopy with blind biopsy of the base of the tongue or diagnostic tonsillectomy since primary tumors, in contrast to the advanced lymph node status, can be small and hardly detectable by clinical examination alone.

A strong correlation between the cytological and histological p16 expression of lymph nodes and their primary tumors could be of decisive importance for determining the localization of the primary tumor. Furthermore, the prognostic information of the FNA could be improved if the predictive value of p16 obtained from the lymph node was high. This would allow correctly distinguishing this biologically and prognostically different subtype of HPV/p16-positive OPSCC from the majority of nicotine- and alcohol-associated HNSCC.

The aim of this study was to (1) analyze the correlation of p16 expression in HNSCC primary tumors and their lymph node metastases, (2) evaluate the feasibility of immunocytochemical (ICC) p16 staining in FNA samples of HNSCC lymph node metastasis, and (3) compare the lymph node IHC with the matching lymph node ICC p16 expression.

Materials and methods

Patient data and specimen characteristics

At the University Hospital in Basel a tissue microarray (TMA) of HNSCC samples of previously untreated patients with HNSCC with complete medical history and follow-up data was constructed [11]. Exclusion criteria for integration were second primary cancer after initial curative therapy, no curative treatment, incomplete clinical data, and biopsy too small to be punched for TMA integration. Therefore, out of more than 600 patients treated in Basel from 1988 to 2003, 365 primary tumor specimens remained to be integrated into the TMA. Of 54 included patients, archival paraffin embedded tissue blocks of a clearly corresponding lymph node metastasis was available for additional integration in the TMA. Finally, for 25 of these 54 patients an archival FNA of the lymph node metastasis was available. Two fine-needle aspiration probes per patient were taken for the initial diagnosis of cancer; one single slide per patient was integrated in our study.

Immunohistochemical p16 analysis

Four-micrometer sections of TMA blocks were transferred to an adhesive coated slide system (Instrumedics, Hackensack, New Jersey) supporting the cohesion of 0.6 mm array elements on glass. Standard indirect immunoperoxidase procedures were used for immunohistochemistry (ABC-Elite, Vector Laboratories, Burlingame, CA) on an automated stainer (BondVR System, Menarini Diagnostics). A monoclonal antibody was used for p16 detection (Clone E6h4, MTM Laboratories AG, Heidelberg, Germany). Optimal staining could be achieved after pretreatment with microwave oven (100 °C 20 min. BondVR buffer, dilution 1/400). Nuclei were counterstained with hematoxylin. The primary antibody was omitted as a negative control. P16 protein expression was scored semi-quantitatively blinded to clinical parameters.

Immunocytochemical p16 analysis in lymphatic FNA

FNA material was smeared on glass slides. Slides were wet fixed and stained with Papanicolaou stain for diagnosis. Papanicolaou stained slides that had adequate amount of representative material were de-stained. Immunocytochemistry was performed utilizing the Leica BOND Max automated stainer (Leica, Nunningen, Switzerland). Slides were pre-treated in citrate buffer for 10 min at 95 °C. The antibody p16 of the CINtec® Cytology kit (Ref 9521, mtm laboratories, Heidelberg, Germany) was used as a primary antibody at a dilution of 1/100. Staining was visualized by diaminobenzidine. Immunocytochemical staining for p16 was regarded as positive if a strong cytoplasmic reaction was observed. The number of stained cells was estimated semi-quantitatively.

Statistical section

Concordance between p16 expression in matched primary tumor and lymph node metastases as well as between histological and cytological expression in both primary tumor and lymph node metastases was assessed using the Kappa statistic (κ) and 95 % confidence intervals (CI). Level of agreement was interpreted according to the following values of κ: >0.8–1.0 excellent, >0.6–0.8 substantial, >0.4–0.6 moderate, >0.2–0.4 fair, and 0–0.2 slight [12]. Percent agreement, namely the number of concordant over total number of cases was additionally evaluated. Analysis was performed using SAS V9.2 (The SAS Institute, Cary, NC).

Results

Patients and clinico pathological parameters

Fifty-four patients were treated with a complete resection of the primary tumor and a neck dissection. Three patients had no lymph node metastasis, 18 were diagnosed with a stage pN1, three were pN2a, 21 were pN2b, and eight were pN2c, and one single patient pN3. Eighteen of the patients had a stage III carcinoma, the remaining 36 a stage IV tumor. The distribution of the most important clinico pathological parameters is shown in Table 1.

Immunohistological p16 expression in the primary tumor and lymph node metastases

Thirteen of 54 primary tumors were p16-positive, eight of these were localized in the palatal tonsil, four in the base of the tongue, and one only in the supraglottic larynx. Metastases in 14 neck specimens were positive for p16 expression. Ten of 13 p16-positive primary tumors showed correlating p16-positive lymph nodes while one positive primary had p16-negative lymph node metastases and two negative primaries had p16-positive lymph node metastases. Correlation of the histological p16 expression in the primary tumor and the neck specimens corresponded precisely; the κ value (95 % CI) of 0.85 represents an excellent matching. The values for sensitivity and specificity were 95.1 and 92.4 %. Results are shown in Table 2.

Correlation of immunocytochemical and immunohistochemical p16 expression in matched lymph node metastases

In 25 of the 54 HNSCC patients, a FNA of the lymph node metastases was taken before treatment. All eight neck metastases stained immunohistologically p16-positive showed a cytological p16 staining. Of the 17 histologically p16-negative lymph node metastases 15 showed negative p16 cytology, whereas two cytological samples were positive. Again, the κ value (95 % CI) of 0.83 represents an excellent correlation. The values for sensitivity and specificity were 88.2 and 100 %. Results are shown in Table 3.

Correlation of immunocytochemical p16 expression in lymph node metastases with immunohistological p16 expression in matched primary tumors

All eight primary tumors stained immunohistologically p16-positive showed p16 staining in the FNA sample. Of the 17 histologically p16-negative primary tumors 15 showed negative p16 cytology and two cytological samples were p16-positive. These results, with a κ value (95 % CI) of 0.75, represent a substantial correlation. Results are shown in Table 4.

Discussion

A correct staging of the neck is of decisive importance for HNSCC patients as lymph node metastases bear the most selective prognostic pretreatment information. Small HNSCC including the stages I and II have a good prognosis while the 5-year-survival rate of advanced stage HNSCC, mainly with involvement of lymph node metastases, lies under 50 %. Furthermore, the lymph node involvement is one of the important treatment selection criteria: while small stage tumors without lymph node involvement are treated by a single modality, surgery or radiotherapy only, advanced tumors with lymph node metastases are treated by combined modalities. Although CT, MRI, and PET-CT lead to significant improvements in the correct clinical neck staging, radiologic imaging depends on the specialist's interpretation and bears limitations [13]. Gold standard for a detection of a true positive cervical lymph node metastasis is a tissue specimen confirming neoplastic cells. The most reliable clinical information about a precise neck staging is achieved by US-controlled FNA of the suspicious lymph nodes [1]. The advantages of this technique including availability, minimal discomfort for the patient, lack of radiation exposure, and low costs are on the other hand faced with its main challenges, such as dependence on the performers' and cytologists' experience as well as the difficulty to reliably detect the deeply localized retropharyngeal lymph nodes. In our institution, we benefit from an excellent cooperation of a highly experienced team of ultrasonographers and cytologists. To our knowledge, we present the first study evaluating p16 expression in the FNA of a lymph node metastasis with the immunohistochemical p16 expression of the corresponding lymph node metastasis and primary HNSCC. Our results are based on aspirated material smeared on glass slides, wet fixed with ethanol in a bedside technique, and stained for diagnosis with Papanicolaou, whereas others report cytological p16 examinations of paraffin-fixed material examined by routine histology [14] or analyzed p16 by in situ hybridization (ISH) [15, 16] or by fluorescence in situ hybridization [17] or PCR [18].

The HPV-positive OPSCC are primarily located in the palatine and lingual tonsils [19]. A biopsy of these primary tumors is frequently connected with discomfort for the patient and requires an examination under general anesthesia. Interestingly, a majority of these HPV-associated OPSCC are reported to present with cervical lymph node metastases [8, 9]. The tumors causing the cervical lymphadenopathy can again be proven by a conventional FNA.

Table 1 Clinico pathological parameters in the 54 patients with head neck squamous cell carcinoma

Number	Age	Sex	Localization	cT	cN	Stage	IHC PT	IHC LN	ICC LN
1	63	M	Hypopharynx	2	2b	4	0	0	0
2	51	M	Hypopharynx	3	2b	4	0	0	0
3	57	M	Hypopharynx	3	1	3	0	0	0
4	53	M	Larynx glottic	2	2c	4	0	0	0
5	73	M	Larynx transglottic	4	2b	4	0	0	0
6	62	M	Oral cavity	2	2b	4	0	0	0
7	63	M	Oral cavity	4	2c	4	0	0	0
8	46	M	Oral cavity	3	2a	4	0	0	0
9	67	F	Oral cavity	3	1	3	0	0	0
10	62	M	Oral cavity	2	2b	4	0	0	0
11	65	M	Oral cavity	2	1	3	0	0	0
12	42	M	Soft palate	4	1	4	0	0	0
13	66	F	Tonsil	2	3	4	0	0	0
14	50	M	Tonsil	3	2b	4	0	0	0
15	56	M	Tonsil	3	2b	4	0	0	10
16	60	F	Tonsil	4	2c	4	0	0	10
17	51	M	Back wall of oropharynx	1	1	3	0	0	×
18	41	M	Base of tongue	1	2b	4	0	0	×
19	64	F	Base of tongue	2	1	3	0	0	×
20	72	M	Base of tongue	2	1	3	0	0	×
21	53	F	Hypopharynx	2	1	3	0	0	×
22	83	M	Hypopharynx	4	0	4	0	0	×
23	47	M	Hypopharynx	2	2b	4	0	0	×
24	63	M	Oral cavity	4	1	4	0	0	×
25	60	M	Oral cavity	4	2b	4	0	0	×
26	70	F	Oral cavity	1	2b	4	0	0	×
27	52	F	Oral cavity	3	1	3	0	0	×
28	29	M	Oral cavity	3	1	3	0	0	×
29	65	M	Oral cavity	2	1	3	0	0	×
30	59	M	Oral cavity	2	2b	4	0	0	×
31	42	M	Oral cavity	2	1	3	0	0	×
32	55	M	Oral cavity	2	1	3	0	0	×
33	59	M	Oral cavity	3	0	3	0	0	×
34	42	M	Oral cavity	4	2b	4	0	0	×
35	37	M	Oral cavity	3	2c	4	0	0	×
36	54	F	Oral cavity	3	0	3	0	0	×
37	54	M	Oral cavity	3	2b	4	0	0	×
38	59	M	Oral cavity	4	2b	4	0	0	×
39	61	F	Oral cavity	4	2b	4	0	0	×
40	74	M	Tonsil	1	2a	4	0	80	×
41	70	M	Base of tongue	2	2c	4	0	100	×
42	58	F	Tonsil	1	2b	4	10	100	50
43	40	M	Base of tongue	2	2b	4	30	0	0
44	57	F	Tonsil	2	2b	4	40	10	30
45	55	M	Base of tongue	3	2b	4	80	100	80
46	64	M	Base of tongue	2	1	3	100	75	×
47	63	M	Larynx supraglottic	4	2c	4	100	90	30
48	60	M	Tonsil	1	1	3	100	90	90

Table 1 continued

Number	Age	Sex	Localization	cT	cN	Stage	IHC PT	IHC LN	ICC LN
49	53	F	Tonsil	2	2b	4	100	100	30
50	54	M	Tonsil	1	2a	4	100	100	30
51	61	M	Base of tongue	2	2c	4	100	100	50
52	56	M	Tonsil	2	1	3	100	100	×
53	68	M	Tonsil	2	1	3	100	100	×
54	50	M	Tonsil	4	2c	4	100	100	×

cT clinical classification of the primary tumor (PT), *cN* clinical classification of the cervical lymph node (LN) metastases, *IHC* immunohistochemistry, *ICC* immunocytochemistry

Table 2 Agreement in histologic p16 expression between matched primary tumor and lymph node metastases in patients with HNSCC

	Histology: lymph node			Percent concordance	κ (95 % CI)
	Negative	Positive	Total		
Histology: primary tumor					
Negative	39 (72.2 %)	2 (3.7 %)	41		
Positive	1 (1.9 %)	12 (22.2 %)	13	94.4 %	0.85 (0.7–1.0)
Total	40	14	54		

Predictive values: sensitivity 95.1 %, specificity 92.4 %

Table 3 Agreement in p16 expression between histologically and cytologically examined matched lymph node metastases in patients with HNSCC

	Cytology: lymph node			Percent concordance	κ (95 % CI)
	Negative	Positive	Total		
Histology: lymph node					
Negative	15 (60 %)	2 (8 %)	17		
Positive	0 (0 %)	8 (32 %)	8	92 %	0.83 (0.6–1.0)
Total	15	10	25		

Predictive values: sensitivity 88.2 %, specificity 100 %

Table 4 Agreement in p16 expression between histologically examined primary tumors and matched cytologically examined lymph node metastases in patients with HNSCC

	Cytology: lymph node			Percent concordance	κ (95 % CI)
	Negative	Positive	Total		
Histology: primary tumor					
Negative	14 (56 %)	2 (8 %)	16		
Positive	1 (4 %)	8 (32 %)	9	88 %	0.75 (0.5–1.0)
Total	15	10	25		

As the prognosis of HPV-positive OPSCC can differ significantly from the HPV-negative HNSCC, information about HPV status of the cytological aspirate is desirable.

The verification of HPV-positive in OSPCC can be made by several techniques, such as ISH or PCR. As it correlates precisely to HPV-positive, overexpression of p16 is suggested as a surrogate marker for HPV 16/18 infection [5, 6]. Several studies have reported a significant impact of p16 expression on survival of OPSCC [7, 9, 19, 20]. These results could be confirmed by two large

multimodal analyses of outcome parameters in OPSCC by Granata [21] and Ang [22], who reported a strong differentiation of OPSCC risk groups by HPV 16 ISH as well as p16 expression, the latter being unspecific for HPV 16 and thus discriminating OPSCC independently of the HPV type.

Ideally, this risk stratifying information should be available before treatment, with minimal technical effort and least discomfort for the patient, which can be achieved by one single small tissue aspirate of a FNA.

We therefore analyzed the p16 expression in 54 HNSCC primary tumors and the matched neck dissection specimens by IHC and correlated these results with matched pretreatment archival FNA by ICC p16 expression. On the histological level, results of p16 expression in the primary and its cervical lymph node metastases matched precisely [κ (95 % CI) 0.85 (0.7–1.0)]; results for sensitivity and specificity were 95.1 and 92.4 %. This correlation confirmed histological results reported by Begum et al. [16]. The reliability of an HPV-related cytological analysis in archival slides had been assessed by Umudum et al. [15]. They showed precisely matching results on HPV DNA ISH in lymph node metastasis aspirates compared with the matching primary OPSCC. ISH is an elaborate and expensive technique available only at a limited number of centers. We therefore chose the evaluation of the ICC p16 protein staining out of four reasons: first, p16 is an accepted surrogate marker for HPV 16-positive [5, 6], second, p16 staining is technically easily performed, third, the interpretation of p16 staining is simple, as 5 % or more cytoplasmatic stained tumor cells are determined to be a positive result and fourth, a p16 immunostaining is much less expensive than ISH. The histological analyses of the neck dissection specimens revealed 8 p16-positive and 17 p16-negative lymph node metastases. All histologically positive lymph nodes were confirmed by a positive p16 ICC. Of the 17 p16-negative biopsies, 15 cytology specimens correlated but two specimens showed false positive results. The percent concordance reached 92 % [κ (95 % CI) 0.83 (0.6–1.0)] and results for sensitivity and specificity were 88.2 and 100 %. The same results were found in the analyses for the p16 expression in the primary HNSCC and the matching cytology aspirates. Again the percent concordance was highly significant. Interestingly, the two false positive ICC slides showed the weakest p16 staining of all aspirates with 10 % positive cells only. All other positive slides showed a strong and clear p16 staining result with at least 30 % positive tumor cells. We considered whether the archival cytological aspirates are more delicate concerning the interpretation of minimally positive tumor samples and whether the cut-off value for p16-positive in cytology smears should be analyzed and determined apart from the histological cut-off applied, which is generally accepted to be 5 % of positive tumor cells. This value is based on a tissue section evaluating hundreds of tumor cells. As cytological aspirates include significantly less tumor cells for evaluation, the precise percentage of p16-positive cells to be reliable for p16-positive of the tumor as a whole becomes debatable. With an elevation of the cut-off for p16-positive to >10 % positive tumor cells in the aspirates a 100 % correct correlation of the FNA with the histological evaluations could be achieved.

All but one of the p16-positive tumors was located in either the palatal or the lingual tonsil. The single p16-positive carcinoma outside the oropharynx was classified as supraglottic laryngeal carcinoma. This classification was made on the basis of the radiological (CT) and clinical examination. At the reevaluation of the archival histological slide, this advanced tumor deeply infiltrated the base of the tongue, leaving its correct origin open for interpretation, as it could just as well be classified as OPSCC of the lingual tonsil infiltrating the supraglottic tissue. Even more, as the p16-positive HNSCC are in the vast majority of oropharyngeal tonsillar origin, the ICC of p16 could optimize the precise determination of tumor localization apart from the precise clinical staging and thorough radiological workup. Including this additional information all OPSCC were correctly localized in the oropharynx by the histological p16 staining of the neck metastases, which correlated precisely with their primary tumors.

Our results suggest that p16-positive of histologically examined neck metastases correlate to the matching primary tumor. Furthermore, cytological p16 staining concordance with the matching lymph node metastasis as well as the primary OPSCC is high and reliable even in archival aspirates. In our view, a cytological analysis of cervical lymph node metastasis bears relevant additional information to the proof of the metastatic origin of the lymph node itself: in addition to the clinical examination and thorough radiologic workup it can facilitate the correct localization of the primary tumor and can help to discriminate biologically different types of OPSCC with their significantly diverse prognosis. Patients with OPSCC without the conventional risk factors, namely smoking and alcohol consumption, and a p16-positive FNA could currently at least benefit from the improved prognostic information.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. de Bondt RB, Nelemans PJ, Hofman PA, Casselman JW, Kremer B, van Engelshoven JM, Beets-Tan RG (2007) Detection of lymph node metastases in head and neck cancer: a meta-analysis comparing US, USgFNAC, CT and MR imaging. Eur J Radiol 64:266–272

2. Bernier J, Domenge C, Ozsahin M, Matuszewska K, Lefèbvre JL, Greiner RH, Giralt J, Maingon P, Rolland F, Bolla M, Cognetti F, Bourhis J, Kirkpatrick A, van Glabbeke M (2004) Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 350:1945–1952
3. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, Kish JA, Kim HE, Cmelak AJ, Rotman M, Machtay M, Ensley JF, Chao KS, Schultz CJ, Lee N, Fu KK (2004) Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med 350:1937–1944
4. Dahlstrand H, Nasman A, Romanitan M, Lindquist D, Ramqvist T, Dalianis T (2008) Human papillomavirus accounts both for increased incidence and better prognosis in tonsillar cancer. Anticancer Res 28:1133–1138
5. Hafkamp HC, Speel EJ, Haesevoets A, Bot FJ, Dinjens WN, Ramaekers FC, Hopman AH, Manni JJ (2003) A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and overexpression of p16INK4A and p53 in the absence of mutations in p53 exons 5-8. Int J Cancer 107:394–400
6. Li W, Thompson CH, O'Brien CJ, McNeil EB, Scolyer RA, Cossart YE, Veness MJ, Walker DM, Morgan GJ, Rose BR (2003) Human papillomavirus positivity predicts favourable outcome for squamous carcinoma of the tonsil. Int J Cancer 106:553–558
7. Rischin D (2010) Oropharyngeal cancer, human papilloma virus, and clinical trials. J Clin Oncol 28:1–3
8. Hafkamp HC, Manni JJ, Haesevoets A, Voogd AC, Schepers M, Bot FJ, Hopman AH, Ramaekers FC, Speel EJ (2008) Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. Int J Cancer 122:2656–2664
9. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Sasaki C, Rimm DL, Psyrri A (2004) Prognostic significance of p16 protein levels in oropharyngeal squamous cell cancer. Clin Cancer Res 10:5684–5691
10. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R (2008) Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 100:407–420
11. Sauter G, Simon R, Hillan K (2003) Tissue microarrays in drug discovery. Nat Rev Drug Discov 2:962–972
12. Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33:159–174
13. Ng SH, Yen TC, Liao CT, Chang JT, Chan SC, Ko SF, Wang HM, Wong HF (2005) 18F-FDG PET and CT/MRI in oral cavity squamous cell carcinoma: a prospective study of 124 patients with histologic correlation. J Nucl Med 46:1136–1143
14. Jannapureddy S, Cohen C, Lau S, Beitler JJ, Siddiqui MT (2010) Assessing for primary oropharyngeal or nasopharyngeal squamous cell carcinoma from fine needle aspiration of cervical lymph node metastases. Diagn Cytopathol 38:795–800
15. Umudum H, Rezanko T, Dag F, Dogruluk T (2005) Human papillomavirus genome detection by in situ hybridization in fine-needle aspirates of metastatic lesions from head and neck squamous cell carcinomas. Cancer 105:171–177
16. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH (2003) Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. Clin Cancer Res 9:6469–6475
17. Uzawa N, Sonoda I, Myo K, Takahashi K, Miyamoto R, Amagasa T (2007) Fluorescence in situ hybridization for detecting genomic alterations of cyclin D1 and p16 in oral squamous cell carcinomas. Cancer 15:2230–2239
18. Pai RK, Erickson J, Pourmand N, Kong CS (2009) p16(INK4A) immunohistochemical staining may be helpful in distinguishing branchial cleft cysts from cystic squamous cell carcinomas originating in the oropharynx. Cancer 25:108–119
19. Fischer CA, Kampmann M, Zlobec I, Green E, Tornillo L, Lugli A, Wolfensberger M, Terracciano LM (2010) p16 expression in oropharyngeal cancer: its impact on staging and prognosis compared with the conventional clinical staging parameters. Ann Oncol 21:1961–1966
20. Lewis JS Jr, Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, El-Mofty SK (2010) p16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. Am J Surg Pathol 34:1088–1096
21. Granata R, Miceli R, Orlandi E, Perrone F, Cortelazzi B, Franceschini M, Locati LD, Bossi P, Bergamini C, Mirabile A, Mariani L, Olmi P, Scaramellini G, Potepan P, Quattrone P, Ang KK, Licitra L (2011) Tumor stage, human papillomavirus and smoking status affect the survival of patients with oropharyngeal cancer: an Italian validation study. Ann Oncol [Epub ahead of print]
22. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML (2010) Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 363:24–35