Cytology and Human Papillomavirus Testing on Cytobrushing Samples From Patients With Head and Neck Squamous Cell Carcinoma

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BACKGROUND: The increasing incidence of human papillomavirus (HPV)-related head and neck squamous cell carcinoma (HNSCC) highlights the need for simple and effective tools to evaluate head and neck lesions and their HPV status. The main objective of the current study was to investigate the association between abnormal cytology and HPV infection, assessed on cytobrushing samples, and histologically confirmed HNSCC. Second, the authors attempted to investigate whether HPV status on cytobrushing samples reflected that of the tumoral tissue. METHODS: A total of 164 samples from HNSCC, nonmalignant lesions, or healthy mucosae of the oral cavity and oropharynx were collected by cytobrushing in PreservCyt solution and evaluated by liquid-based cytology and Linear Array HPV genotyping test. All the findings from the cytologic samples were compared with those from the corresponding histologic samples. RESULTS: Patients with abnormal cytology had a significantly higher risk of having an HNSCC (odds ratio [OR], 9.18; 95% confidence interval [95% CI], 3.27-26.49). The association was stronger for oral cancer (OR, 10.86; 95% CI, 2.51-51.06) than oropharyngeal cancer (OR, 8.45; 95% CI, 1.62-49.82). HPV positivity in the oropharyngeal cytobrushing was associated with a nearly 5-fold higher risk of having abnormal cytology (OR, 4.57; 95% CI, 1.57-13.57) as well as histologically proven oropharyngeal cancer (OR, 5.09; 95% CI, 1.09-31.61). Comparing the HPV status on cytologic and corresponding histologic samples from patients with HNSCC, we found that 90.4% of the cases were concordant (kappa, 0.796). CONCLUSIONS: Abnormal brushing cytology is strongly associated with a diagnosis of HNSCC, whereas HPV positivity on cytobrushing samples is only associated with oropharyngeal cancer. HPV testing on cytobrushing samples represents a valid option for the assessment of HPV infection in patients with oropharyngeal cancer.

Cancer 2014;120:3477-84. © 2014 American Cancer Society.

KEYWORDS: cytology, human papillomavirus, head and neck, squamous cell carcinoma.

INTRODUCTION

The incidence of human papillomavirus (HPV)-related head and neck squamous cell carcinoma (HNSCC) has been significantly increasing over the last few years, as demonstrated by a growing number of studies conducted in Europe and North America.1,2 HPV, particularly the HPV type 16, has been found to be etiologically associated especially with SCC of the oropharynx.3,4 Notably, HPV-positive cancers differ from the HPV-negative ones with regard to response to treatment and survival, with HPV infection being a favorable prognostic marker.5-7 Therefore, HPV testing is acquiring a growing significance for patients with HNSCC, particularly those with oropharyngeal SCC, and there is an increasing demand for simple, rapid, and effective methods for sample collection and the evaluation of HPV infection. Importantly, diagnostic tissues may be very scant in patients with small primary tumors, making it difficult to perform further analyses such as HPV testing. In addition, there is a growing interest in the use of cytology for the evaluation of HN lesions.8-11 In fact, although histology represents the gold standard in HN pathology, cytologic sampling and liquid-based cytology12 might represent valuable tools for an initial assessment of the lesions, allowing for the simultaneous evaluation of morphology and HPV infection. By rapidly providing a preliminary characterization of the lesions, cytology may also help shorten the patient management time.

Recently, cytology has been proposed as a noninvasive, painless, and low-cost screening tool for the detection of HN lesions in asymptomatic individuals at risk of HPV infection and HNSCC. In particular, it might reveal morphologic abnormalities in subjects with oral/oropharyngeal HPV infection but with no visible lesions.13,14 However, to establish a...
possible role for cytology in the secondary prevention of HNSCC, the area to be sampled when obvious lesions are not present has to be defined and large population-based studies are needed to assess indicators of cytology accuracy. Differently, a candidate tool with a possible clinical use may be preliminarily evaluated on a selected series of samples with or without the lesion of interest. To the best of our knowledge, there are very few studies to date regarding the use of exfoliative cytology in the evaluation of patients with HNSCC, and even less on the concurrent assessment of HPV status on a large series of samples. On the basis of these assumptions, the objective of the current study was to investigate whether cytologic sampling by cytobrushing may represent a useful tool for the evaluation of HN lesions and their HPV status. In particular, we investigated whether abnormal cytology was associated with a diagnosis of SCC of the oral cavity (OC) and oropharynx (OP). An additional objective of the current study was to evaluate whether HPV infection observed on cytobrushing was associated with a diagnosis of OPSCC and whether HPV testing performed on cytologic samples provided reliable information regarding the HPV status of the tumoral tissue. To this end, morphologic findings and HPV testing on cytology were compared with the corresponding findings on histologic samples, which were considered to be the gold standard.

MATERIALS AND METHODS

Study Population
All participants were recruited at the Department of Otolaryngology and Head Neck Surgery of the Regina Elena National Cancer Institute from January 2012 to February 2013. Two populations were enrolled: 1) consecutive patients who consulted the physician involved in the study for diagnostic evaluation of OC or OP lesions; and 2) individuals with no clinically evident lesions who were participating in a nested study aimed at investigating OC and OP HPV infection in healthy individuals.

All participants provided informed consent. The study was approved by the Ethics Committee of the Regina Elena National Cancer Institute (CE/485/12). Patients were managed according to the criteria and recommendations of the National Comprehensive Cancer Network (version 2.2013) for the diagnosis and treatment of patients with signs and symptoms of HNSCC.

Cytologic and Histologic Sample Collection
For patients with OC/OP lesions, the cytologic sample was collected during the first consultation and before any treatment by rubbing the lesional mucosa with a cytobrush (Hologic, Pomezia, Italy) 3 to 5 times. Immediately after, a biopsy was obtained that was fixed in formalin using standardized methods.

The participants with no clinically evident lesions were subjected to cytologic sampling only, using a single cytobrush. Because in the HN region there are no indications regarding a specific area to be sampled, OC samples were collected from the hard palate, the gums, the front two-thirds of the tongue, and the floor of the mouth below the tongue. OP samples were collected from the soft palate, the base of the tongue, and the tonsils. The cytobrush was swirled vigorously in 20 mL of PreservCyt (Hologic) and stored at 4°C. The same physician collected the cytologic samples from both the individuals with OC/OP lesions and those with no clinically evident lesions.

Cytologic and Histologic Evaluation
Liquid-based cytology slides were prepared using the ThinPrep 2000 Processor (Hologic) and stained according to the Papanicolaou method. Slides were interpreted by 2 experienced cytopathologists blinded to all other findings and an adjudicated final report was established. Morphology was interpreted based on the criteria described by Silverman and mainly in agreement with previous reports. Cytologic findings were classified into 4 categories: inadequate (material too scanty to be representative), negative, atypical, and positive. Samples containing malignant atypias were classified as positive (Fig. 1). Whenever abnormalities did not clearly fulfill the malignant criteria, the morphology was classified as atypical. Atypical and positive reports were all considered to be abnormal cytology and were compared with the cases with a negative report, which were considered as normal cytology.

The biopsies were processed according to standard procedures and independently examined by 2 pathologists blinded to the other findings. An adjudicated final diagnosis was established following the World Health Organization classification. Tumor grade was classified as well, moderate, or poor. Tumor size was evaluated according to the 2009 International Union Against Cancer/TNM classification. The histopathologic findings were considered as the gold standard for the confirmation of the presence and type of OC/OP lesions.

HPV DNA Testing
All the samples were tested for HPV during the routine activity of the study laboratory as they arrived from the Otolaryngology Department. Total nucleic acids were
extracted from 5 to 10 mL of the PreservCyt samples using the AmpliLute Liquid Media Extraction kit (Roche Diagnostics, Milan, Italy), and from 4 μm ×10 μm sections of the formalin-fixed paraffin-embedded (FFPE) tissue using the DNeasy Blood and Tissue Kit (Qiagen, Milan, Italy) as previously described. Ten μL of extract was screened for HPV DNA using the Linear Array HPV Genotyping Test (Roche Diagnostics), which is able to detect 37 mucosal HPV genotypes. Due to the possible false-negative results of the test on FFPE samples, the Linear Array-negative FFPE cases were further analyzed by the Inno-LiPA HPV Genotyping Extra kit (Innogenetics, Pomezia, Italy), which detects 28 mucosal HPV genotypes. HPV carcinogenic risk (high risk [HR] or low risk [LR]) was classified in accordance with International Agency for Research on Cancer indications and as previously described.

Statistical Analyses
Correlation of cytologic findings (abnormal cytology) with histologic diagnoses (SCC) was estimated by calculating the raw agreement and Cohen kappa values. For the assessment of the association between the outcomes of interest (cytology, HPV infection, and histology), 2×2 contingency tables were used to calculate odds ratios (OR) and 95% confidence intervals (95% CIs). Whenever the association of a variable with histology was evaluated, only those cases with a histologic diagnosis were considered. Therefore, patients with no clinically evident lesions were excluded from these analyses because they did not undergo a biopsy. For purpose of analysis, the cases with a histologic diagnosis were classified in 2 categories: those cases with a diagnosis of HNSCC and those cases with a diagnosis other than cancer.

The agreement of the HPV test results on the cytologic and histologic paired samples (HPV negative vs positive regardless of the specific HPV genotype[s] identified) was measured by the Cohen kappa. The histologic cases that were negative on Linear Array were retested by the Inno-LiPA assay and, in the case of positivity, the result of the latter assay was considered for the comparison. All statistical analyses were conducted using the SPSS statistical package (version 17.0; SPSS Inc, Chicago, Ill).

RESULTS

Study Population
The study population included 164 individuals (62 women and 102 men), 119 of whom had a clinically evident OC/OP lesion, whereas 45 were had no OC/OP lesions. Overall, 164 cytologic samples were collected from all the study participants. In addition, 119 biopsies were obtained from the individuals with OC/OP lesions;
74 corresponded to a diagnosis of HNSCC whereas 45 had a diagnosis other than HNSCC (10 cases of hyperplasia, 3 cases of mild dysplasia, 22 cases of papilloma, 5 cases of hyperkeratosis, and 5 cases with no relevant morphologic alterations). With regard to the HNSCC cases, 31 were of the OC (74% of them from the mobile tongue) and 43 were of the OP (51% from the tonsils and 39% from the base of the tongue). Histopathologic grading was known for 58 cases: 1 case (1.7%) was graded as well, 32 cases (55.2%) were graded as moderately, and 25 cases (43.1%) were graded as poorly differentiated. Tumor size, which was available for 44 patients, was as follows: 15 patients had T1 disease (34.0%), 13 patients had T2 disease (29.6%), 2 patients had T3 disease (4.6%), and 14 patients had T4 disease (31.8%).

The median age of the cancer patients was 66 years (interquartile range [IQR], 56 years-72 years), whereas that of the patients with a diagnosis other than HNSCC and of those with no OC/OP lesions was 42 years (IQR, 37 years-60 years) and 38 years (IQR, 31 years-51 years), respectively.

**Comparison Between Cytologic and Histologic Findings**

Among the 164 cytologic samples, 13 were inadequate (7.9%), 3 of which came from the 74 specimens obtained from patients with cancer (4.0%). Table 1 shows the distribution of the cytologic reports by anatomic site and presence/type of lesion. Notably, all the positive reports corresponded to cancer cases. The raw agreement between cytology and histology considering all cases was 75.5% (Cohen kappa, 0.47; 95% CI, 0.30-0.60). For the OC site, the raw agreement was 76.9% (Cohen kappa, 0.53; 95% CI, 0.30-0.76), whereas the corresponding figure for the OP site was 74.1% (Cohen kappa, 0.39; 95% CI, 0.12-0.67).

Table 2 shows the association between cytologic results and histologic diagnoses for the 106 patients with a biopsy and a valid cytologic report. Patients with cytologic abnormalities had a 9-fold increased risk of having histologically confirmed HNSCC (OR, 9.18; 95% CI, 3.27-26.49). The same strong association was observed for the OC and OP, separately. Notably, the false-negative reports were found among the cases of T1 disease, except for one case that corresponded to a T2 classification. It is also interesting to note that the false-negative rate decreased as the histopathologic grading increased (data not shown).

**HPV Test Results in the Cytologic Samples**

All 164 cytologic samples were analyzed for HPV infection and no invalid HPV test result was observed. Regarding the HNSCC cases, we found that 2 of 31 patients with OCSSC (6.4%) were HPV positive, harboring LR HPV type 62 and HR HPV type 51, respectively, whereas 25 of 43 patients with OPSCC (58.1%) were HPV positive, all of whom had HR HPV types. In particular, 22 of 25 patients (88.0%) had the HPV type 16, alone or as a coinfection, and the other 3 patients (12.0%) were infected by HPV type 33. HPV was detected in 4 of 30 patients with nonmalignant OC lesions (13.3%), all of whom were infected with LR HPV types, and in 3 of 10 patients with nonmalignant OP lesions (30.0%), 2 of whom were infected by HR (types 16 and 73, respectively) and 1 by LR (HPV 6 and 61) HPV types. Finally, HPV was demonstrated in 3 of 50 samples (6.0%) collected from the clinically healthy patients, all of whom were positive for LR HPV's only.

**HPV Test Results in Paired HNSCC Cytologic and Histologic Samples**

To verify whether the HPV test results obtained on cytologic samples reflected HPV status in the tumor tissue, we...
performed the HPV test on the corresponding FFPE samples that had enough residual material for the analysis: 27 OPSCCs and 25 OCSCCs. The OPSCC cases allowed us to verify whether cytologic HPV testing accurately identified HPV-positive tumors, given that OPSCCs are expected to be for the most part HPV positive. Conversely, OCSCC cases, given their low HPV prevalence, allowed us to establish whether cytologic HPV testing properly identified HPV-negative tumors without detecting any infections unrelated to the tumor. No invalid result was obtained. As reported in Table 3, 47 of the 52 cases analyzed were concordant for the HPV status, demonstrating an excellent raw agreement (90.4%) and a good kappa value (0.796 [standard error, 0.121]; \( P < .001 \)). In particular, 32 of the 34 HPV-negative cancer tissues (94.1%) were also HPV negative in the cytologic sample, and 15 of the 18 HPV-positive biopsies (83.3%) were concordantly positive in the corresponding cytologic sample. Notably, 14 OPSCCs that were positive for HPV type 16 in the cytobrushing samples were tested on the histologic sample and HPV type 16 infection was confirmed for all cases. It is interesting to note that the only 2 HPV type 16-positive OPSCC samples that presented with a coinfection in the cytologic sample were found to be infected by the HPV type 16 alone in the histologic material.

Discordant HPV test results were only observed in 5 cases. Two cases were HPV positive in the cytologic sample and negative in the matching histologic specimen. They corresponded to the 2 OCSCC cases that were positive for HPV types 51 and 62, respectively, in the cytobrushing samples. The corresponding histologic samples tested negative both by the Linear Array and the Inno-LiPA assay. Only in the case that was positive for HPV type 51 did we manage to establish any lack of agreement, although we could not draw any definite conclusions concerning the presence of HPV type 62 because this type is not included in the spectrum of genotypes detected by the Inno-LiPA assay. Three cases were HPV negative in the cytobrusching sample and HPV16 positive in the histologic sample. They all represented OPSCC cases with a negative cytologic report.

**Association Between Cytologic and Histologic Findings With HPV Infection in the Cytologic Sample**

Table 4 shows the association between HPV infection detected in the cytobrushing sample with abnormal cytology and with histologically confirmed HNSCC by tumor site. These associations were significant only for the OP specimens. In fact, HPV positivity in the OP cytobrushsing sample was associated with a nearly 5-fold higher risk of abnormal cytology (OR, 4.57; 95% CI, 1.57-13.57). In addition, patients found to be HPV positive in the OP cytologic sample were significantly more likely to have a diagnosis of OPSCC compared with those with an HPV-negative cytobrushing specimen (OR, 5.09; 95% CI, 1.09-31.61). When we considered the OC for comparison, we found that HPV positivity was not significantly associated either with abnormal cytology or histologically confirmed SCC.

**DISCUSSION**

In the current study, we evaluated whether cytology and HPV testing on cytobrushing specimens may be valuable tools for the preliminary characterization of HN lesions in symptomatic individuals. Despite the increasing number of reviews that emphasize the expanding role of cytology in HN pathology,\(^3,6\) to our knowledge there are very few studies to date that examine OC/OP brushing cytology. At present, cytology, as well as HPV testing, is used

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**TABLE 2.** Association Between Abnormal Cytology With Histologically Confirmed HNSCC for the 106 Samples With an Oral/Oropharyngeal Biopsy and a Valid Cytologic Report

| Cytology  | HNSCC No. (%) | OR (95% CI) |
|-----------|---------------|-------------|
| Overall   |               |             |
| Normal    | 17/43 (39.5)  | 1.00        |
| Abnormal  | 54/63 (85.7)  | 9.18 (3.27-26.49) |
| Oral cavity |            |             |
| Normal    | 6/23 (26.1)   | 1.00        |
| Abnormal  | 23/29 (79.3)  | 10.86 (2.51-51.06) |
| Oropharynx|               |             |
| Normal    | 11/20 (55.0)  | 1.00        |
| Abnormal  | 31/34 (91.2)  | 8.45 (1.62-49.82) |

**TABLE 3.** Agreement of HPV Status on Cytologic and Histologic Paired Samples From 52 Patients With HNSCC \(^a\)

| HPV Status on Histology | HPV Status on Cytology | Negative, No. (%) | Positive, No. (%) |
|-------------------------|------------------------|-------------------|------------------|
| Negative                | 32 (94.1)              | 3 (16.7)          |
| Positive                | 2 (5.9)                | 15 (83.3)         |
| Total                   | 34 (100)               | 18 (100)          |

**TABLE 4.** Association Between Abnormal Cytology With Histologically Confirmed HNSCC for the 106 Samples With an Oral/Oropharyngeal Biopsy and a Valid Cytologic Report

**Abbreviations:** HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus.\(^a\) Agreement: 90.4%; expected agreement: 52.8% (kappa, 0.796; standard error, 0.121 [\( P < .001 \)].
mainly on fine-needle aspirates of metastatic sites to identify occult primary HN tumors and to provide information regarding the primary tumor site.20,21 To the best of our knowledge, the current study represents one of the few studies in which samples obtained by cytobrushing were simultaneously analyzed by liquid-based cytology and HPV testing. Cytologic samples were collected from individuals who presented for a clinical consultation because of OC/OP lesions. In addition, samples were also obtained from subjects with no clinically evident OC/OP lesions to validate the sampling method, assess sample quality and quantity, and evaluate the cytologic presentation of OC/OP cells in healthy individuals. In fact, because oral brushing cytology is not routinely used in clinical practice, samples collected from these subjects were used to have a firm understanding of how normal OC and OP epithelial cells appear.

We found that only a limited number of samples were morphologically inadequate due to poor cellularity (7.9%). Nonetheless, all the samples produced a valid HPV test result. In a similar study, nearly all the cytobrushing samples were evaluable for HPV (99%) and nearly 85% of them were morphologically interpretable.9

Cytology performance indicators could not be assessed due to the characteristics of the current study, which was not appropriate to obtain these measures. In fact, accuracy indicators are strongly influenced by the characteristics of the study group, including disease prevalence.22 In our investigation, disease prevalence was extremely high, because the study sample was composed of a large number of patients with HNSCC. In spite of this major limitation of the current study, we were able to evaluate the strength of the association between abnormal cytology and histologically confirmed SCC. Regarding this analysis, it is worth noting that the group of patients with a histologic diagnosis other than HNSCC was heterogeneous and also included 3 cases of mild dysplasia. Because in clinical practice it is important to define the nature of a lesion (i.e., to distinguish between malignant and nonmalignant ones), it is our opinion that the inclusion of dysplasias in the group of cases with no malignancy was not detrimental. In fact, although this surely affected the measure of the association between cytologic abnormalities and HNSCC, it possibly determined an underestimation of the association. Nonetheless, we observed that patients with abnormal cytology were 9-fold more likely to have histologically confirmed HNSCC. As it is to be expected, the association was stronger for OCSCC (OR, 10.86) than OPSCC (OR, 8.45). It is likely that the higher accessibility of the OC made it possible for us to obtain diagnostic material more easily than for the OP. In fact, the base of the tongue and the tonsillar crypts are less accessible to sampling by brushing.

HPV testing of HNSCC, particularly of OPSCC, is acquiring a growing interest due to the prognostic value of HPV status, with HPV-positive HNSCC demonstrating a more favorable prognosis and an increased sensitivity to ionizing radiation.5-7 The use of accurate testing methods is essential for the assessment of tumor HPV status, which can be determined on a variety of clinical specimens, including cytologic material obtained by brushing/scraping, fine-needle aspiration, and oral rinses.9,21,23,24 Previously, it has been shown that oral rinses are more efficient in providing adequate material for HPV testing compared with brushing/scraping.25 However, based on the findings of the current study, we can state that cytobrushing is also capable of providing a suitable specimen for HPV detection. In addition, oral rinses may not ensure the sampling of the tumoral area, and in fact they are not sensitive for malignancy23 and might lead to the detection of an HPV infection that is unrelated to the cancer. Conversely, cytobrushing, which can be specifically targeted to the lesion, increases the collection of site-specific representative cells. Importantly, because cytologic samples are easily collected before the biopsy, they offer the chance to obtain information regarding the HPV status of the lesion very quickly.

| HPV          | Abnormal Cytology | OR (95% CI) | HNSCC No. (%) | OR (95% CI) |
|--------------|-------------------|-------------|----------------|-------------|
| Oropharynx   |                   |             |                |             |
| Negative     | 18/55 (32.7)      | 1.00        | 18/29 (62.1)   | 1.00        |
| Positive     | 20/29 (69.0)      | 4.57 (1.57-13.57) | 25/28 (89.3)  | 5.09 (1.09-31.61) |
| Oral cavity  |                   |             |                |             |
| Negative     | 29/61 (47.5)      | 1.00        | 29/56 (51.8)   | 1.00        |
| Positive     | 3/6 (50.0)        | 1.10 (0.14-8.89) | 2/6 (33.3)    | 0.47 (0.04-3.60) |

Abbreviations: 95% CI, 95% confidence interval; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; OR, odds ratio.
without waiting for the histologic diagnosis that must be obtained before the FFPE sample can be used for molecular analyses.

For the investigation of HPV infection in patients with HNSCC, both direct and indirect methods have been used. These include polymerase chain reaction (PCR)-based assays, in situ hybridization, HPV mRNA detection, signal amplification techniques, and p16 immunostaining.26-29 However, to our knowledge, there is no consensus regarding the most appropriate method. In the present investigation, we used the Linear Array, a PCR-based assay, which also made it possible for us to obtain genotype information. Furthermore, this assay has been shown to have a good performance for HPV detection on cytologic samples as well as on FFPE tissues.16,30

Importantly, the comparison between the HPV status in the cytologic sample and that in the corresponding histologic specimen, both of which were tested with PCR-based methods, showed a good agreement (kappa, 0.79), thereby providing evidence that HPV status in cytobrushing is representative of that in the tumor. Conversely, previous studies that used either brushings or oral rinses for the evaluation of HPV infection demonstrated only a weak concordance with the tumor HPV status.31 However, the majority of previous studies did not investigate HPV on cytologic and histologic samples with the same technique, possibly introducing a bias due to the different assays used.9,29 Regarding the discordant cases, HPV was found in 2 cytologic samples from patients with OCSCC, both of which were morphologically positive for cancer cells, whereas the corresponding FFPE specimens were HPV negative. Therefore, in these 2 cases, PCR revealed HPV infections that were likely to be unrelated to the cancer. The results of the current study regarding FFPE OCSCC cases confirm the available data, which indicate that these tumors rarely harbor HPV DNA.32 Three OPSCC cases were HPV negative in the cytologic specimen but HPV positive in the corresponding cancer tissue. However, they were also morphologically negative, possibly due to the difficulty in reaching the tumor site (base of tongue and glossoepiglottic vallecula) or to the small tumor size (2 cases were classified as T1 and 1 case was classified as T2).

Importantly, we observed that the presence of HPV DNA in the cytologic sample was strongly associated with abnormal cytology for the OP but not for the OC. A previous study has reported similar findings in terms of an association between HPV infection and cytologic alterations.9 In addition, HPV infection was found to be strongly associated with a diagnosis of OPSCC, thereby confirming previous reports.31,33 As is to be expected due to the low prevalence of HPV in OCSCC, no significant association between HPV positivity and this cancer was observed. These findings indicate that HPV testing on cytologic samples may be useful only for those patients with OP lesions.

Finally, the results of the current study demonstrated that abnormal cytology is significantly associated with a diagnosis of HNSCC, particularly of the OC. Conversely, HPV positivity on cytobrushing is strongly associated only with a diagnosis of OPSCC, although abnormal cytology per se suggests the presence of OPSCC more strongly than HPV positivity. Moreover, HPV testing on cytobrushing provides reliable information regarding the HPV status of the tumor and therefore represents a valid option for the assessment of HPV infection in patients with OPSCC. We believe the data from the current study support the idea that sampling of the OC and OP by cytobrushing, a minimally invasive and low-cost technique, is feasible and in the majority of cases provides enough material for a morphologic evaluation and always provides adequate specimens for HPV testing.

FUNDING SUPPORT
No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES
The authors made no disclosures.

REFERENCES
1. Marur S, D’Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol. 2010;11:781-789.
2. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011;29:4294-4301.
3. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev. 2005;14:467-475.
4. Chung CH, Gillison ML. Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. Clin Cancer Res. 2009;15:6758-6762.
5. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363:21-35.
6. Licitra L, Perrone F, Bossi P, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol. 2006;24:5630-5636.
7. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008;100:261-269.
8. Holmes BJ, Westra WH. The expanding role of cytopathology in the diagnosis of HPV-related squamous cell carcinoma of the head and neck. Diagn Cytopathol. 2014;42:85-93.
9. Fakhry C, Rosenthal BT, Clark DP, Gillison ML. Associations between oral HPV16 infection and cytopathology: evaluation of an
oropharyngeal “pap-test equivalent” in high-risk populations. Cancer Prev Res (Phila). 2011;4:1378-1384.

10. Krane JF. Role of cytology in the diagnosis and management of HPV-associated head and neck carcinoma. Acta Cytol. 2013;57:117-126.

11. Jarboe EA, Hunt JP, Layfield LJ. Cytomorphologic diagnosis and HPV testing of metastatic and primary oropharyngeal squamous cell carcinomas: a review and summary of the literature. Diagn Cytopathol. 2012;40:491-497.

12. Navone R, Burlo P, Pich A, et al. The impact of liquid-based oral cytology on the diagnosis of oral squamous dysplasia and carcinoma. Cytomorphology. 2007;18:356-360.

13. Gillison ML, Broustian T, Pickard RK, et al. Prevalence of oral HPV infection in the United States, 2009-2010. JAMA. 2012;307:693-703.

14. Du J, Nordfors C, Ahrlund-Richter A, et al. Prevalence of oral human papillomavirus infection among youth, Sweden. Emerg Infect Dis. 2012;18:1468-1471.

15. Silverman SJ. Oral cavity. In: Bibbo M, ed. Comprehensive Cytology. Philadelphia: WB Saunders; 1991:399-408.

16. Donà MG, Ronchetti L, Giuliani M, et al. Performance of the linear array HPV genotyping test on paired cytological and formalin-fixed, paraffin-embedded cervical samples. J Mol Diagn. 2013;15:373-379.

17. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 100B. A Review of Human Carcinogens: Biological Agents. Lyon, France: IARC; 2011.

18. Donà MG, Palamara G, Di Carlo A, et al. Prevalence, genotype diversity and determinants of anal HPV infection in HIV-uninfected men having sex with men. J Clin Virol. 2012;54:185-189.

19. Shoukri MM. Measures of Interobserver Agreement and Reliability. 2nd ed. Chapman & Hall/CRC Biostatistics Series. Boca Raton, FL: CRC Press; 2011.

20. Zhang MC, El-Mofty SK, Davila RM. Detection of human papillomavirus-related squamous cell carcinoma cytologically and by in situ hybridization in fine-needle aspiration biopsies of cervical metastasis: a tool for identifying the site of an occult head and neck primary. Cancer. 2008;114:118-123.

21. Begum S, Gillison ML, Nicot TL, Westra WH. Detection of human papillomavirus-16 in fine-needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. Clin Cancer Res. 2007;13:1186-1191.