Journal of Clinical Virology 85 (2016) 22–26

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Human papillomavirus detection in fine needle aspiration cytology of lymph node metastasis of head and neck squamous cell cancer

Robert P. Takes[a], Johannes H.A.M. Kaanders[b], Carla M.L. van Herpen[c], Matthias A.W. Merkx[d], Pieter J. Slootweg[e], Willem J.G. Melchers[f,*]

[a] Department of Otolaryngology and Head and Neck Surgery, Radboud University Medical Center, Nijmegen, The Netherlands
[b] Department of Radiation Oncology, Radboud University Medical Center, Nijmegen, The Netherlands
[c] Department of Medical Oncology, Radboud University Medical Center, Nijmegen, The Netherlands
[d] Department of Oral and Maxillofacial Surgery, Radboud University Medical Center, Nijmegen, The Netherlands
[e] Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands
[f] Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form 12 October 2016
Accepted 14 October 2016

Keywords:
Human papilloma virus
Cytology
Head and neck
Squamous cell carcinoma
Unknown primary tumor

ABSTRACT

Background: Currently, testing on HPV in oropharyngeal squamous cell carcinoma (OPSCC) is performed on histological material. However, in a certain percentage of the cases who present with lymph node metastases no primary tumor can be identified and only fine needle aspiration cytology (FNAC) is available for analysis.
Objectives: Purpose of this study was to assess HPV status on FNAC and to validate it using histological material of the same patients.
Study design: Patients with cervical metastasis from OPSCC or cancer of an unknown primary tumor (CUP), diagnosed between 2007 and 2012 were included. In 6 of the 47 patients, no primary tumor could be identified. HPV detection and genotyping was performed in both FNAC slides scrapings and formalin fixed paraffin embedded (FFPE) histological material from the same patients, using the HPV SPF_{10}-$LiPA_{25}$ assay. HPV PCR analysis on FFPE material was considered the reference standard for HPV status of each case.
Results: Compared with HPV negative cases (n = 22), significantly more HPV positive cases (n = 25) presented initially with cervical metastasis (27% vs 56% respectively; p = 0·047). The HPV PCR assay on FNAC material showed a high sensitivity (96%; 95% CI 86.6–97.4) and specificity (100%; 95% CI 85.1–96.7) using the reference standard of HPV PCR analysis on FFPE material of the same patients.
Conclusion: In this study, testing on HPV in FNAC of cervical lymph node metastases of SCC is validated. It provides a valuable alternative for testing of HPV on histological material from patients with oropharyngeal squamous cell carcinoma or cancer of an unknown primary tumor.

1. Background

In the last decade it became evident that viral integration of high-risk human papillomavirus (hrHPV) types, especially HPV type 16, is an important factor in the oncogenesis of oropharyngeal squamous cell carcinoma (OPSCC) [1,2]. Through overexpression of the HPV oncoproteins E6 and E7, as a result of disruption of E2 after HPV-DNA integration into the host DNA, the host tumor-suppressor proteins p53 and retinoblastoma (Rb) are degraded. This subsequently leads to p53 dysfunction and $p16^{INK4A}$ upregulation [3,4]. This mechanism is responsible for the distinct molecular, clinical and pathological entity of HPV-related OPSCC as compared with HPV-unrelated tumors, which are associated with alcohol and smoking habits [1,5]. The prevalence of HPV-related OPSCC is rising and reported with a reported wide range between 20% and 90% [6,7].

It has become evident that HPV-positive OPSCCs patients have a better survival outcome with a significant reduction in mortality in comparison with patients with HPV negative tumors. This is thought to be partly due to a favorable response to ionizing

Abbreviations: CUP, cancer of an unknown primary tumor; FNAC, fine needle aspiration cytology; HPV, Human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma.

* Corresponding author at: Department of Medical Microbiology, Radboud University Medical Center, PO Box 9101, 6500, HB, Nijmegen, The Netherlands.
E-mail address: willem.melchers@radboudumc.nl (W.J.G. Melchers).

http://dx.doi.org/10.1016/j.jcv.2016.10.008

radiation and chemotherapy regimes, attributed to an intact p53 apoptosis pathway [8,9]. Therefore, HPV status has become a major prognostic factor in patients with OPSCC.

Despite extensive diagnostic workup, including a thorough clinical and endoscopical examination with the taking of biopsies and the use of several imaging techniques, two to five percent of cases who present with neck nodes containing metastatic SCC remain with an unidentified primary site of origin [10,11]. This is thought to be due to a possible involution of the primary tumor or a slow growth rate of the primary tumor as compared to their metastases, resulting in a very small and undetectable primary tumor with a more advanced N-stage [5]. In particular in case of a primary location in the tonsillar crypts and the base of the tongue such a small primary tumor may remain undetected. So it may be hypothesized that at least a part of the patients with cancer of an unknown primary tumor (CUP) could in fact suffer from small undetected OPSCCs. Therefore, important diagnostic and prognostic information can be expected to be provided by the HPV status of the cancer cells in CUP, as it may point towards the most likely site of origin of the primary tumor in case of HPV-positivity, namely the oropharynx. Testing on HPV in this category of patients with CUP to localize the primary tumor site in the oropharynx is an accepted concept now.

Currently, the HPV status of OPSCC is commonly determined through the accepted algorithm of HPV detection by PCR combined with p16 immunohistochemical (IHC) staining on histological tumor material [12,13]. However, in case of CUP, no histological material of a primary tumor is available for HPV analysis. If HPV status can be reliably established by testing on material obtained by fine needle aspiration cytology (FNAC) of metastatic lymph nodes, it can be used to locate the likely origin of the primary HNSCC in CUP without the necessity to obtain material from a neck dissection specimen.

To validate if testing on cytological material matches with testing on histological material, we evaluated HPV-PCR analysis in FNAC from cervical lymph node metastasis and compared it to the golden standard of HPV-PCR on histological material from the same patients. Although HPV analysis in FNAC material has been reported previously, the validation using histological material of the same patients has not been done before as far as we are aware of.

2. Study design

2.1. Case selection

FNAC samples obtained from metastatic lymph nodes of patients diagnosed with OPSCC or CUP of the head and neck diagnosed between 2007 and 2012 were identified and retrieved from the files of the Department of Pathology of the Radboud University Medical Center in Nijmegen, The Netherlands. Formalin fixed paraffin embedded (FFPE) histological material taken from the primary tumor or metastatic lymph node of the corresponding cases was retrieved as well.

Cases were included if the FFPE material was both HPV and p16 IHC negative or both HPV and p16 IHC positive. p16 IHC was considered positive in cases that showed both diffuse and intense nuclear and cytoplasmic staining in almost all tumor cells. Exclusion criteria were: a second primary tumor in the head and neck region to avoid uncertainty about the relationship between primary tumor and metastasis, insufficient cytological material for processing, and previous exposure of the neck to radiotherapy.

Patient medical records were reviewed to document the primary tumor site, metastatic lymph node location and general histopathological characteristics of the samples.

2.2. Tissue preparation and DNA purification

The detection and genotyping of HPV was performed on scraped FNAC material from archival slides. The FNAC slides were reviewed for determining tumor cell density and representativeness. After soaking in xylene to remove the coverglass, the cytological material on the slides was completely scraped off and transferred into 200 μL phosphate buffered saline (PBS) suspension. Isolation and purification of the DNA was performed with MagNa pure 96 (Roche Molecular Diagnostics). The purified DNA was diluted in 50 μL in elution buffer (Roche Molecular Diagnostics) and stored in −20 °C until further processing by PCR. In the process, internal extraction and amplification controls were included to ensure a valid en reliable procedure.

DNA was isolated from FFPE tissue sections (4 μM) with the EZ1 robot (Qiagen, Germany, with the DNA tissue kit of Qiagen) according to standard procedures [14] and used for PCR analysis. A negative water control was included with each batch of 10 samples.

2.2.1. HPV-DNA detection and typing

Broad-spectrum HPV-DNA amplification was performed using a short-PCR-fragment assay (HPV SPF_{10}-$LiPA_{25}$, version 1; Labo Biomedical Products B.V, Rijswijk, Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame of HPV genotypes, as described by Melchers et al. [14] HPV genotyping was performed using a cocktail of 9 conservative probes in a micro titer hybridization assay, the DNA enzyme immunoassay (DEIA). The samples positive for HPV by DEIA were then analyzed with the line probe assay (LiPA25) by reverse hybridization with type-specific probes for HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74. The LiPA strips were visually inspected and interpreted following the standardized reference guide. Phocine Herpesvirus (PhHV) was used as an internal control for amplification.

2.2.2. Statistical analysis

Pathological and clinical characteristics were assessed using a contingency table χ^2 tests. By applying cross tabulation sensitivity, specificity, positive predictive value and negative predictive value were calculated (and 95% confidence intervals (CI)) for final HPV 16 classifications compared with the 'golden standard'. Cohen κ was used to analyze the measure of agreement between histological and cytological HPV analysis by means of SPF-10 assay. Statistical analysis was conducted using the statistical software package SPSS 20.0 (IBM corporation, 2011).

3. Results

3.1. Clinical and pathological characteristics

In our institute approximately 100 OPSCCs and 10 CUPs are diagnosed and treated annually. Based on the availability of histological material already tested on HPV (in routine practice) and the availability of complementary FNAC material of these cases a selection was made. The inclusion criteria were met by 47 cases. In total, 25 HPV 16 positive cases and 22 HPV negative cases were included for analysis. Compared with HPV negative cases (n = 22), significantly more HPV positive cases (n = 25) presented initially with a metastasis to a regional lymph node (27.3% vs 56% respectively; p = 0.047).

The characteristics of both groups are presented in Table 1. The mean ages of both cohorts was similar (mean 57.7 years vs. 58.4 years; p = 0.4) as well as the distribution of gender in the two groups (p = 0.36). No clear difference in primary location between the two groups was observed (p = 0.48). Twenty cases presented initially with SCC in the neck only. In six out of the total of 47 cases (13%) no