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The role of HPV on the risk of second primary neoplasia in patients with oropharyngeal carcinoma

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ABSTRACT

Objectives: It has been reported that patients with HPV-positive oropharyngeal cancer (OPC) have a lower risk of appearance of second primary neoplasm (SPN) than HPV-negative OPC patients. The aim of our study was to analyze the risk of developing SPN in a large group of patients with OPC according to HPV status in the primary tumor.

Materials and methods: We included 412 OPC patients treated at our center from 1991 to 2014 for which the HPV DNA positivity was evaluated by PCR in available tumor specimens. HPV DNA positive samples were further tested for HPV E6∗I mRNA detection and/or $p16^{INK4a}$ immunohistochemistry. We estimated the incidence of SPN in all cancer sites and in cancer sites related to tobacco and alcohol consumption according to the HPV status in the primary tumor.

Results: Fifty-one (12.4%) out of 412 OPCs included in the study were HPV-related. Five-year SPN-free survival for HPV-negative versus HPV-positive OPC patients was 57.0% and 89.0% ($P < 0.001$), respectively. Corresponding estimates for 10-year SPN-free survival were 35.2% versus 78.5% ($P < 0.001$). When restricting the analyses to tobacco/alcohol-related SPNs, the corresponding survival rates where 62.0% versus 97.6% ($P < 0.001$) and 42.2% versus 97.6%, ($P < 0.001$), for 5-year and 10-year survival rates, respectively. HPV status and previous toxic habits might allow classifying patients regarding the risk of tobacco/alcohol-related SPNs.

Conclusion: HPV-related OPC patients have a significant lower risk of SPN development, particularly in those locations related to tobacco use or alcohol consumption.

Introduction

Patients with head and neck squamous cell carcinoma (HNSCC) have a high risk of developing a second primary neoplasm (SPN), located predominantly in sites epidemiologically related with the classic carcinogenic mechanisms (i.e. tobacco and alcohol consumption) that promoted the appearance of the index tumor such as lung, esophagus and again head and neck [1]. Besides the traditional HNSCC pattern of carcinogenesis associated with tobacco use and alcohol consumption, high-risk human papilloma virus (HPV) has been recognized as an emerging carcinogen, particularly in the oropharynx [2]. These viral mediated tumors are epidemiologically, clinically and biologically different from other HNSCC promoted by the carcinogenic action of tobacco and alcohol [3–5].

Studies evaluating the HPV status in OPC patients with serological markers [6], immunohistochemical $p16^{INK4a}$ staining [7] or in situ hybridization (ISH) [8,9] have reported a decreased incidence in SPN among HPV-positive OPC patients. Moreover, several studies have reported lower rates of SPN among patients with OPC than among patients with other head and neck tumors location, probably related to the HPV etiological origin [10,11].

Furthermore, over the last few years, population studies based on the Surveillance, Epidemiology and End Results (SEER) data

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base, a program of the National Cancer Institute that collects cancer incidence and survival data from approximately 28% of the U.S. population [12], have found a decreased incidence in second synchronic [13] and metachronic [14] neoplasm in patients having an index tumor located in the oropharynx. Global increasing trends of HPV-related OPC [15], and the lower risk of SPN among these patients might explain the observed decrease in the development of SPN after an oropharyngeal index tumor.

The aim of our study was to analyze the risk and associated factors of developing SPN in OPC patients regarding HPV status. HPV positivity was evaluated by HPV DNA PCR and other HPV oncogenic biomarkers such as E6∗I mRNA or $p16^{INK4a}$.

Material and methods

Patient selection

We carried out a study in a single institute cohort of OPC patients from which the clinical data, tumor characteristics and follow-up was collected prospectively [16].

Eligible subjects for the present report were those with a pathologically confirmed primary oropharyngeal invasive squamous cell carcinoma treated at our center between 1991–2014, with available formalin-fixed paraffin-embedded (FFPE) sample of the tumor.

During the study period, 835 patients with OPC were treated at our center. Four hundred and twelve were eligible and included in the analysis. Non-eligible subjects were mainly those diagnosed at other centers and referred to our hospital for treatment from whom the FFPE was not available (n = 423).

HPV status assessment

HPV status of OPC patients diagnosed between 1991–2012, was evaluated as part of a retrospective international study, as previously reported [17]. Briefly, at least five sections were obtained for each paraffin block. First and last sections were used for histopathological evaluation, and the in-between ones for DNA and mRNA HPV testing, and $p16^{INK4a}$ immunohistochemistry (IHC). HPV DNA testing was performed in all cases with SPF-10 PCR/DEIA/LiPA25 system. All HPV-DNA positive samples underwent RNA extraction and E6∗I mRNA detection by type specific RT-PCR, and $p16^{INK4a}$ expression assessment. HPV status in this retrospective component was based on mRNA positivity as gold standard for HPV oncogenic involvement. To note that HPV E6∗I mRNA was detected in all cases showing an overexpression of $\geqslant$70% of stained cells.

For patients diagnosed between 2013 and 2014, HPV status was prospectively evaluated as part of the clinical routine based on HPV DNA detection by real time PCR amplification following instructions of CLART HPV 2 by GENOMICA (GENOMICA S.A.U, Madrid, Spain). The HPV-DNA positive samples were also evaluated for $p16^{INK4a}$ immunohistochemistry under the manufacturer's standards (p16 antibody BCAM 16123 on autostainer platform and enVision Flex-linker (DAKO) visualization system). A diffuse pattern of more than 50% of the tumor cells with moderate or high intensity was required to define p16 immunopositive protein expression. HPV status in this prospective component was based on $p16^{INK4a}$ overexpression.

Variable considerations

The definition of the main outcome variable, SPN, was based on the criteria by Warren and Gates [18]. Accordingly, each SPN should be confirmed histologically, the metastatic origin of a previous tumor should be ruled out, and there should be no submucous connection between the tumors. Head and neck, lung, bladder and esophagus tumors were considered SPN related to tobacco and/or alcohol consumption.

HPV-relatedness in the primary OPC was considered when the sample was HPV DNA positive and either mRNA (retrospective cohort) or $p16^{INK4a}$ (prospective cohort) were positive. TNM 7th edition was used to classify the subsite location of OPC tumors: anterior wall, including base of tongue and vallecula; lateral wall, including tonsil, tonsillar fossa and pillars, and glossotonsillar sulci; superior wall, including the soft palate and uvula; and posterior wall [19]. According to the interaction between tobacco and alcohol consumption, we created a combined variable of toxic consumption with three categories: no consumption; moderate consumption (<20 cigarettes/day and/or <80 gr alcohol/day); and severe consumption ($\geqslant$20 cigarettes/day and/or $\geqslant$80 gr alcohol/day). The median follow-up time of patients included in the study was 5.1 years (standard deviation 4.3 years).

Statistical analyses

We carried out a comparative analysis between patients included in the study according to OPC HPV status. Overall SPN and tobacco/alcohol-related SPN frequency were analyzed stratified by age, gender, toxics consumption, local and regional disease extension, tumor differentiation grade, and HPV status. Univariate comparison of patient characteristics between groups was performed using the Chi-square test or Fisher's exact test for categorical variables, as appropriate.

SPN-free survival was estimated according to HPV status. These analyses were performed for overall SPN and for those SPN epidemiologically associated with tobacco and alcohol as previously explained. Actuarial survival was estimated using the Kaplan-Meier method. The log-rank test was used to compare survival functions. We classified the patients according to the risk of developing SPN in locations related to tobacco and alcohol with a recursive partitioning analysis (classification and regression tree model), taking into account the OPC HPV status and toxic consumption. A multivariate analysis was conducted using Cox's proportional hazard regression. In these analyses we included the following variables: sex, age, T category, N category, OPC subsite, toxics consumption, and HPV status. Toxics and HPV status were included in the models either separately and combined in a single variable following results of the recursive partitioning analysis.

The ethics committees of both Hospital de Sant Pau and the Catalan Institute of Oncology approved the study protocols. The study conforms to the principles outlined in the Declaration of Helsinki.

Results

Fifty-seven of the 412 OPC (13.8%) were HPV DNA positive. HPV16 was the genotype most frequently found, in 50 of the 57 HPV DNA-positive samples (87.7%). Other genotypes detected were HPV18 (three cases), and HPV33-35-51-58 (one case each). HPV E6∗I mRNA was evaluated on 43 HPV DNA positive samples of which 39 (90.7%) were positive (from the retrospective cohort). In 14 HPV DNA positive samples for which mRNA detection was not assessed (from the prospective cohort), $p16^{INK4a}$ IHC was performed and 12 (85.7%) were considered positive.

HPV-positive OPC was defined as tumor with HPV DNA positivity and at least another positive biomarker, E6∗I mRNA (n = 39) or $p16^{INK4a}$ IHC (n = 12). Finally, fifty-one (12.4%) of the OPC cases were considered HPV-positive.