Oral Oncology 78 (2018) 137–144

Contents lists available at ScienceDirect

Oral Oncology

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

Double positivity for HPV-DNA/p16^{ink4a} is the biomarker with strongest diagnostic accuracy and prognostic value for human papillomavirus related oropharyngeal cancer patients

Marisa Mena[a,b,*,1], Miren Taberna[a,c,d,1], Sara Tous[a,b], Sandra Marquez[a], Omar Clavero[a,b], Beatriz Quiros[a,b], Belen Lloveras[e], Maria Alejo[f], Xavier Leon[g,h], Miquel Quer[g], Silvia Bagué[i], Ricard Mesia[c,d,j], Julio Nogués[k], Montserrat Gomà[l], Anton Aguila[m], Teresa Bonfill[n], Carmen Blazquez[o], Marta Guix[p,q], Rafael Hijano[r], Montserrat Torres[a], Dana Holzinger[s], Michael Pawlita[s], Miguel Angel Pavon[a], Ignacio G. Bravo[t], Silvia de Sanjosé[a,u], Francesc Xavier Bosch[a,b], Laia Alemany[a,u,*]

[a] Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
[b] Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain
[c] Department of Medical Oncology, Catalan Institute of Oncology (ICO), ONCOBELL, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
[d] University of Barcelona, Barcelona, Spain
[e] Department of Pathology, Hospital del Mar, Barcelona, Spain
[f] Department of Pathology, Hospital General de l'Hospitalet, Barcelona, Spain
[g] Department of Otorhinolaryngology, Hospital de Sant Pau, Barcelona, Spain
[h] Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain
[i] Department of Pathology, Hospital de Sant Pau, Barcelona, Spain
[j] Department of Medical Oncology, Catalan Institute of Oncology (ICO), Badalona, Barcelona, Spain
[k] Department of Otorhinolaryngology, Hospital Universitari Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
[l] Department of Pathology, Hospital Universitari Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
[m] Department of Otorhinolaryngology, Hospital Universitari Parc Taulí, Sabadell, Barcelona, Spain
[n] Department of Medical Oncology, Hospital Universitari Parc Taulí, Sabadell, Barcelona, Spain
[o] Department of Pathology, Hospital Universitari Parc Taulí, Sabadell, Barcelona, Spain
[p] Cancer Research Program, IMIM, Hospital del Mar, Barcelona, Spain
[q] Department of Medical Oncology, Hospital del Mar, Barcelona, Spain
[r] Department of Otorhinolaryngology, Hospital del Mar, Barcelona, Spain
[s] Division of Molecular Diagnostics of Oncogenic Infections, Infection, Inflammation and Cancer Program, German Cancer Research Center (DKFZ), Heidelberg, Germany
[t] Laboratory MIVEGEC (UMR CNRS 5290, IRD 224, UM), French National Center for Scientific Research (CNRS), Montpellier, France
[u] Epidemiology and Public Health, Centro de Investigación Biomédica en Red: Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain

ARTICLE INFO

Keywords:
Human papillomavirus
Oropharyngeal cancer
Prognosis markers
Diagnostic accuracy
Survival

ABSTRACT

Background: The etiologic role of human papillomaviruses (HPV) in oropharyngeal cancer (OPC) is well established. Nevertheless, information on survival differences by anatomic sub-site or treatment remains scarce, and it is still unclear the HPV-relatedness definition with best diagnostic accuracy and prognostic value.
Methods: We conducted a retrospective cohort study of all patients diagnosed with a primary OPC in four Catalonian hospitals from 1990 to 2013. Formalin-fixed, paraffin-embedded cancer tissues were subjected to histopathological evaluation, DNA quality control, HPV-DNA detection, and p16^{INK4a}/pRb/p53/Cyclin-D1 immunohistochemistry. HPV-DNA positive and a random sample of HPV-DNA negative cases were subjected to HPV-*E6*I* mRNA detection. Demographic, tobacco/alcohol use, clinical and follow-up data were collected. Multivariate models were used to evaluate factors associated with HPV positivity as defined by four different HPV-relatedness definitions. Proportional-hazards models were used to compare the risk of death and recurrence among HPV-related and non-related OPC.
Results: 788 patients yielded a valid HPV-DNA result. The percentage of positive cases was 10.9%, 10.2%, 8.5%

* Corresponding authors at: Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), IDIBELL, Av. Gran Via de L'Hospitalet 199-203, 08908 L'Hospitalet de Llobregat, Barcelona, Spain.
E-mail addresses: mmena@iconcologia.net (M. Mena), lalemany@iconcologia.net (L. Alemany).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.oraloncology.2018.01.010
Received 19 October 2017; Received in revised form 15 January 2018; Accepted 17 January 2018
Available online 20 February 2018
1368-8375/

and 7.4% for $p16^{INK4a}$, HPV-DNA, HPV-DNA/HPV-*E6*I* mRNA, and HPV-DNA/$p16^{INK4a}$, respectively. Being non-smoker or non-drinker was consistently associated across HPV-relatedness definitions with HPV positivity. A suggestion of survival differences between anatomic sub-sites and treatments was observed. Double positivity for HPV-DNA/$p16^{INK4a}$ showed strongest diagnostic accuracy and prognostic value.

Conclusions: Double positivity for HPV-DNA/$p16^{INK4a}$, a test that can be easily implemented in the clinical practice, has optimal diagnostic accuracy and prognostic value. Our results have strong clinical implications for patients' classification and handling and also suggest that not all the HPV-related OPC behave similarly.

Introduction

About a decade ago the International Agency for Research on Cancer (IARC) established high-risk *Human papillomavirus 16* (HPV16) as a cause of oropharyngeal carcinoma (OPC) [1]. Since then, increasing amount of information on the role of HPVs in OPC has been generated. The IARC estimates that approximately 29,000 new HPV-related OPC cases occur every year, corresponding to 31% of the worldwide number of the overall incident OPC cases [2]. These estimates, as well as previous meta-analyses assessing the quantitative contribution of HPV, found high geographic heterogeneity in HPV-attributable fractions (AFs) of OPC, ranging from less than 20% in some world regions, 24% in Southern Europe to more than 60% in North America [3,4]. This low HPV-AF for OPC in Southern Europe has been recently confirmed in two recent studies conducted by our group [5,6].

HPV-related OPC differs at clinical, epidemiological and molecular level to OPC caused by classic risk factors (i.e. tobacco and alcohol) [7]. The consistent observation of improved survival and better response to treatment of HPV-related OPC has stirred up the state-of-the-art of their management. Indeed, several clinical trials of de-escalation treatments are under evaluation, aiming to achieve better results with less treatment-associated comorbidities [8]. However, the biological rationale underlying these strategies remains poorly understood, and most of schemes are extrapolated from HPV-negative OPC trials. Importantly, around 20% of HPV-related patients still fail to treatment despite its good prognosis [7].

Diagnosis algorithms for HPV-related OPC are still under development. HPV-DNA detection alone is not sufficient to classify an OPC as HPV-driven since the presence of HPV-DNA could reflect a transient or non-related infection rather than a genuine HPV-driven oncogenic process [9–11]. Additionally, the detection of high cellular $p16^{INK4a}$ expression by immunohistochemistry (IHC) is the most widely implemented technique in the clinical setting, but is not specific for HPV activity in these tumours [12,13]. Indeed, it has been demonstrated that patients with $p16^{INK4a}$ high expression but HPV-DNA-negative OPC show a significantly less favourable survival than patients with $p16^{INK4a}$ high expression and HPV-DNA-positive tumours [14,15], indicating that $p16^{INK4a}$ high expression alone may not accurately classify HPV-related OPC patients. The combination of HPV-DNA detection and $p16^{INK4a}$ IHC is starting to be recommended to diagnose HPV-related OPCs [15]. Nevertheless, there is still limited information about the accuracy and prognostic value of this combination of biomarkers.

It is imperative to identify the best HPV-relatedness definition for HPV causality and prognosis in OPC. This is a prerequisite to provide a sound approach to study differences in survival of HPV-related OPC by factors such as anatomical sub-site [16,17] and by treatment [18].

In an attempt to elucidate these gaps, we conducted a study in OPC to assess the association of different HPV-relatedness definitions with patients' overall survival (OS) and progression-free survival (PFS), stratified by anatomical sub-site or treatment.

Methods

Study design and population

We designed a retrospective cohort study of all patients diagnosed with a primary OPC in four hospitals of Catalonia from 1990 to 2013 (Catalan Institute of Oncology-ICO-Hospital Universitari de Bellvitge, Hospital de Sant Pau, Hospital del Mar and Hospital Parc Taulí). Protocols were approved by the ethics committee of each participating hospitals.

Cancer cases were identified from medical records/pathology reports of the centers of origin. We included cases that fulfilled the following criteria: to be diagnosed with primary invasive cancer of the oropharynx (any histology; codes from the International Classification of Diseases for Oncology version 3: C01.9, C02.4, C05.1, C05.2, C09, C10, C14.2), and to have access to medical records on demographic and clinical information.

From all eligible cases, we reviewed medical records of the patients and accessed information on demographics, smoking and alcohol consumption, clinical and follow-up data; and formalin-fixed paraffin embedded (FFPE) tumour samples from the diagnosis previous to treatment when available.

In order to assess potential carryover HPV contamination at the local level, we additionally included a set of control samples selected by local investigators (5% of the number of cases evaluated, corresponding to tissue samples of patients with diagnoses non- related with HPV processed in the same laboratory).

FFPE blocks processing and histopathological evaluation

All specimens processing was centralized at ICO. FFPE blocks were re-embedded whenever necessary. First and last sections were used for histopathological evaluation after hematoxylin and eosin (H&E) staining. Two in-between sections were used for HPV-DNA testing, genotyping and *E6*I* mRNA detection; four additional slides were obtained to assess expression of cellular proteins by IHC. A block was classified as "adequate" for HPV testing if invasive cancer was observed in the two H&E stained sections of the specimen. Pathology review was performed blind with respect to the original local diagnosis and followed a pre-established algorithm for diagnostic consensus involving three pathologists, as reported elsewhere [5]. Pathological classification was based on the World Health Organization pathological criteria for head and neck cancer [19].

FFPE blocks were processed under strict conditions of pre/post polymerase chain reaction (physical separation), and blank paraffin blocks were systematically tested in parallel to serve as sentinels for contamination as previously published [20].

HPV-DNA detection and genotyping

The detailed methods used for HPV-DNA detection and genotyping have been reported elsewhere [21]. Briefly, we used a PCR with the consensus primers SPF_{10} PCR and a DNA enzyme immunoassay (DEIA) to test for the presence of HPV-DNA. Virus genotyping was performed using reverse hybridization line probe assay (LiPA25_v1) on all samples testing positive for viral DNA, targeting 25 HPV types with different oncogenic risk (Laboratory Biomedical Products Rijswijk, The Netherlands). DNA quality was evaluated in all HPV-DNA negative samples by testing for the *tubulin*-β gene (21). All DEIA and LiPA25_v1 assays were performed at ICO.