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Predictors of oropharyngeal cancer survival in Europe

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ABSTRACT

Objectives: HPV16-positive oropharyngeal cancer (OPC) patients experience better outcomes compared to HPV16-negative patients. Currently, strategies for treatment de-escalation are based on HPV status, smoking history and disease stage. However, the appropriate cut-point for smoking and the role of other non-clinical factors in OPC survival remains uncertain.

Materials and Methods: We examined factors associated with OPC outcome in 321 patients recruited in a large European multi-center study. Seropositivity for HPV16 E6 was used as a marker of HPV16 positive cancer. Hazard ratios (HR) and confidence intervals (CI) were estimated using Cox proportional models adjusted for potential confounders.

Results: Overall 5-year survival following OPC diagnosis was 50%. HPV16-positive OPC cases were at significantly lower risk of death (aHR = 0.51, 95% CI: 0.32–0.80). A significant effect on OPC survival was apparent for female sex (aHR 0.50: 95% CI: 0.29–0.85) and being underweight at diagnosis (aHR: 2.41, 95% CI: 1.38–4.21). A 10 pack year smoking history was not associated with overall survival. Higher stage at diagnosis appeared as the only factor significantly associated with OPC recurrence (aHR: 4.88, 95% CI: 2.12–11.21).

Conclusion: This study confirms that HPV16 status is an independent prognostic factor for OPC survival while

Abbreviations: AJCC, American Joint Committee on Cancer; ARCAGE, the Alcohol Related Cancers and Genetic susceptibility in Europe study; CI, confidence intervals; HPV, human papillomavirus; HR, hazard ratios; OPC, oropharyngeal cancer

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female sex lowers risk of death and being underweight at diagnosis increases the risk of death. Smoking was not an independent predictor of OPC survival.

Introduction

Cancers arising in the oral cavity and pharynx have an estimated global burden of 442,760 incident cases and 241,458 deaths each year [1]. Tobacco smoking and alcohol consumption explain nearly 70% of these cancers [2,3]. Infection by Human PapillomaVirus (HPV), specifically type 16 (HPV16) causes a subset of cancers, particularly those arising at the tonsil, oropharynx, soft palate and base of the tongue (collectively referred to as oropharyngeal cancers-OPC) [4]. Further, HPV16-positive (HPV16+) OPC is described to be epidemiologically, molecularly and clinically distinct from HPV16-negative (HPV16-) OPC [5]. The increasing incidence of OPC in several Western countries is attributed to the increasing HPV16+ fraction [6–9].

Since HPV16+ OPC patients experience better survival outcomes compared to HPV16- patients, alternative staging has been recommended [10]. However, recurrence remains a concern and it is presently unclear which patients may benefit from de-intensified treatment. Clinically, HPV status is ascertained based on HPV DNA and p16 expression or p16 expression alone. HPV status in combination with disease stage and patient smoking history (based on a 10 or 20 pack year cut off) has been suggested to classify patients into prognostic groups and to identify candidates for de-escalation of treatment [10,11]. This scheme has rarely been verified. Further, the appropriate cut-point for pack years of smoking remains uncertain. In addition, the role of other non-clinical risk factors in OPC survival is not fully understood.

To address these knowledge gaps, we tested 321 oropharyngeal tumors in a large series of well characterized European patients for HPV16 serology. In addition, HPV16 DNA, p16 expression were tested in the corresponding tumor tissues when available (n = 198). Applying rigorous protocols of sample processing; we aimed to evaluate the role of HPV16 and other risk factors in predicting OPC survival and recurrence.

Methods

This analysis was based on cases from the European Alcohol Related Cancers and Genetic susceptibility in Europe (ARCAGE) study, conducted across 10 countries in Europe using a standardized protocol [12]. Briefly, over 2000 incident cases of the oral cavity, pharynx, larynx, esophagus and matched controls were recruited during 2002 to 2005. This analysis included squamous cell carcinoma of ICD-O diagnoses C01, C02.4, C05.1- C05.2, C09, C10. All participants underwent personal interviews to record lifestyle exposures. All cases were histologically or cytologically confirmed primary cancers, and cancer stage was ascertained based on the sixth edition of the staging atlas developed by the American Joint Committee on Cancer (AJCC). Tobacco use was broadly categorized as ever or never smokers, ever smokers were defined as individuals who smoked any tobacco product at least once a week for a year. Pack years were calculated for all types of tobacco smoking based on cigarette equivalents. Ever drinkers were those who reported ever consumption of any alcoholic beverage and the consumption of all types of alcoholic beverages was estimated and the total frequency was expressed in terms of drinks of alcohol per day [13]. A weighted composite score of oral hygiene and dental care was constructed as described previously [14] and included denture wear, age at start of denture-wearing and gingival bleeding. A weighted dental care score was also constructed by combining the frequency of tooth cleaning, use of toothpaste, toothbrush or dental floss and frequency of dentist visits, where the maximum score of eight reflected poor dental care. Body mass index was calculated based on weight measured at the time of recruitment. BMI ranging from 18.5 to 25.0 was considered normal, below 18.5 underweight while > 25.0 was considered overweight. Informed consent was obtained from all participants in the study and the study was approved by the ethical review boards at the participating centers.

Pretreatment serum samples were tested for HPV antibodies using the bead-based multiplex serology method [15,16]. We have previously shown that HPV16 E6 antibody is a highly specific marker of HPV16+ OPC with false-positive rates less than 1% [15,17–19]. In addition, other published reports have demonstrated a high sensitivity and specificity for HPV16 E6 serology with false negatives rates of 5–10% based on the definition of gold standard, with stringent definitions having improved rates [20,21]. Here for comparison, 198 available paraffin-embedded OPC tumor blocks were tested based on p16 expression and HPV DNA and compared with HPV16 E6 serology. p16 expression was qualitatively evaluated using the CINtec Histology $P16^{INK4a}$ Kit (9511, mtmlabs) following manufacturer's instructions. Expression was scored based on the percentage and intensity of nuclear or cytoplasmic staining. A combined score of 4 or greater was considered positive for $p16^{INK4a}$ overexpression [15,22]. We have previously demonstrated that this scoring system remains comparable to the more widely used percentage of nuclear and cytoplasmic staining cutoff [15,23–25]. HPV genotyping was performed using the Type-Specific E7 PCR bead-based multiplex assay (TS-E7-MPG, IARC, France) to detect all high-risk HPV types (HPV16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68a, -68b, -73, and -82) and three low-risk HPV types (HPV6, -11, and -70). Briefly, the reporter fluorescence was quantified using Luminex reader 200 (Luminex Corporation, Austin, TX), and cutoffs were computed by adding 5–1.1 multiplied by the median background value expressed as median fluorescence intensity [15,26]. Given that HPV serology was available on all cases and the previously demonstrated high sensitivity and specificity against the tumor HPV16 status, we defined a HPV+ tumor as HPV16 E6 antibody positive.

The participants of this study were initially recruited during 2002–2005. Subsequently, a one-time retrospective follow up was conducted between 2012 and 2015 to obtain last known vital status (alive, death or lost to follow-up) and date of last contact. Mortality data including cause and date of death were obtained from at least two information sources for 75% of cases, and one source for the remaining 25%. In Prague and Aviano follow-up was completed by review of medical charts alone. In all other centers, medical chart reviews together with information from population-based registries at the regional or national level were used. In Athens, Barcelona and Manchester physicians were contacted to obtain patient outcome information, while in Oslo, Zagreb and Glasgow cancer registries were consulted. In Bremen, Turin, Padova and Dublin mortality registries were examined. End of follow-up was defined as the date of last confirmed contact, vital status at censor, or date of death (if applicable). Over 96% of OPC patients' recruited in the study have complete follow-up. Overall survival (all-cause mortality) was evaluated using Cox proportional hazard models, predictors were explored for OPC overall and stratified by stage, sex and HPV16 status. Multivariate cox proportional hazards models were used to estimate HR and 95% CI for HPV16 E6 serology, sex, age, smoking status, alcohol use, dental care, BMI, stage while additional adjustment for the center of recruitment was performed. Mortality was also explored using Kaplan Meier curves. The joint effects on survival were considered by combining cofactors in interaction models. Recurrence data were available on all 321 cases,