Human Papillomavirus Prevalence in Invasive Penile Cancer and Association with Clinical Outcome

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Abbreviations and Acronyms

DSS = disease specific survival

FFPE = formalin fixed paraffin embedded

HPV = human papillomavirus

hrHPV = high risk human papillomavirus

LVI = lymphovascular invasion

PCR = polymerase chain reaction

SCC = squamous cell carcinoma

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Purpose: The incidence of penile cancer is increasing, and is suggested to be explained by changes in sexual practice and increased exposure of men to sexually transmitted high risk human papillomavirus infection. In penile cancers from a Dutch population treated in 1963 to 2001 we found a high risk human papillomavirus prevalence of about 30%. In this study we assessed the prevalence of high risk human papillomavirus-DNA in a more recent, contemporary penile cancer cohort and its association with patient survival.

Materials and Methods: High risk human papillomavirus-DNA presence was assessed by GP5+6+ polymerase chain reaction in 212 formalin fixed, paraffin embedded invasive penile tumor specimens of patients treated between 2001 and 2009. The 5-year disease specific survival was calculated using the Kaplan-Meier method with the log rank test and Cox regression.

Results: High risk human papillomavirus-DNA was detected in a subset of penile cancer cases (25%, 95% CI 19–31). HPV16 was the predominant type, representing 79% (42 of 53) of all high risk human papillomavirus infections. The 5-year disease specific survival in the high risk human papillomavirus negative group and the high risk human papillomavirus positive group was 82% and 96%, respectively (log rank test p=0.016). Adjusted for stage, grade, lymphovascular invasion and age, human papillomavirus status was still prognostic for disease specific survival (p=0.030) with a hazard ratio of 0.2 (95% CI 0.1–0.9).

Conclusions: High risk human papillomavirus-DNA was observed in a quarter of penile cancer cases. No relevant increase in high risk human papillomavirus prevalence in recent decades was observed. The presence of high risk human papillomavirus-DNA in penile cancer confers a survival advantage.

Key Words: penile neoplasms; carcinoma, squamous cell; human papillomavirus 16; survival

The etiology of penile cancer is multifactorial, with smoking, phimosis and poor hygiene commonly associated with this tumor.[1] Other risk factors include number of sexual partners and a history of genital warts or other sexually transmitted diseases.[2] At least some of these risk factors are related to infection with human papillomavirus. In circumcised men HPV prevalence is lower than in uncircumcised men,[3] and penile cancer is

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rare in populations that routinely practice circumcision.[4]

The reported proportion of penile cancer associated with high risk HPV types ranges from 30% to 100%,[1,5–7] depending on the population studied, the methods used for HPV detection and/or histological subtypes analyzed. In the Dutch population (1963 to 2001) we found approximately 30% of penile cancers to be associated with hrHPV.[8–10] Patients with hrHPV positive tumors also had a survival advantage compared to those with hrHPV negative tumors. However, other studies concerning the association between HPV status and patient survival report inconsistent results.[6,11–13] As such, the exact role of HPV as a prognostic factor in penile cancer remains unclear. Moreover the incidence of penile cancer is increasing, and is suggested to be explained by changes in sexual practice and exposure of men to sexually transmitted HPV infection.[14] Recent studies on head and neck SCC have shown an increasing incidence of hrHPV associated subtypes in recent decades in several Western countries including the Netherlands.[15–17] It is conceivable that the percentage of hrHPV penile cancers may have increased over time as well.

In this study we assess the prevalence of hrHPV-DNA in invasive penile cancer in a contemporary Dutch cohort and its association with patient survival. A large series of penile cancer cases from 2001 to 2009 was analyzed for hrHPV-DNA presence by GP5+6+ PCR, and the association between HPV status and patient survival was assessed.

MATERIALS AND METHODS

Study Population

Our institutional cohort comprised 487 patients diagnosed with penile cancer between 2001 and 2009. To obtain hrHPV-DNA data of patients with primary invasive cancer treated between 2001 and 2009, FFPE tissue blocks were retrieved from the archives of the Department of Pathology at the Netherlands Cancer Institute for additional testing. Patients who were initially treated for their primary tumor elsewhere, those treated neoadjuvantly or those with carcinoma in situ were excluded from analysis. For 212 patients sufficient material of the primary invasive tumor was left for HPV-DNA testing. The use of clinical material was in compliance with the respective institutional ethical regulations for surplus material[18] and approved by the institutional translational research board.

All cases were (re)staged according to the TNM 2009 classification for penile cancer and had not received preoperative radiotherapy or chemotherapy. The clinical and pathological characteristics were prospectively kept in our institutional penile cancer database. Followup until 2013 provided information concerning node positivity, disease status and disease specific mortality.

Sample Preparation, Histopathology and hrHPV-DNA Detection and Typing

Sample preparation and hrHPV-DNA detection and typing have been described before.[8–10] For each FFPE specimen a series of consecutive 5 μm sections were cut under safety measures to avoid cross-contamination. The first and last sections were stained with hematoxylin-eosin for histopathology, including confirmation of tumor presence, subtyping and grading according to Broders.[19] In-between sections were collected in a reaction vessel for DNA extraction and subsequent PCR analyses.

Detection of hrHPV on the DNA extracts from FFPE sections was performed by GP5+/6+ PCR enzyme immunoassay using a cocktail of 14 hrHPV types (ie HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) as previously described.[8–10] Beta-globin PCR was performed on each DNA extract as a quality control. hrHPV positive samples were subsequently genotyped by bead based array on the Luminex platform.[20]

Statistical Analyses

Differences in patient characteristics between the hrHPV positive and negative groups were tested for statistical significance. DSS was defined as the time since surgery until death from penile cancer, penile cancer metastasis or complications related to penile cancer treatment. Patients alive at the end of followup were censored. DSS rates were calculated using the Kaplan-Meier method, with the log rank test assessing equality of distributions. Multivariable analysis of survival was performed using the Cox proportional hazards model. All statistical analyses were performed with SPSS® (v20.0) and R version 3.0.2 (http://www.r-project.org/), with 2-sided $p < 0.05$ considered statistically significant.

RESULTS

HPV Prevalence

Overall 53 of 212 (25%, 95% CI 19–31) penile carcinoma cases were positive for hrHPV-DNA, with 42 (79%) containing HPV16, 4 (8%) HPV33, 3 (6%) HPV18, 2 (4%) HPV45, 1 (2%) HPV31 and 1 (2%) HPV52.

Clinicopathological Characteristics

Clinicopathological variables of patients stratified by tumor HPV status are presented in table 1. Patients in the hrHPV positive group tended to have smaller tumors than those in the hrHPV negative group. In the hrHPV negative group 45% of tumors were well differentiated compared to 17% in the hrHPV positive group. In terms of tumor subtypes, no differences in the distribution of SCC vs other subtypes were found between HPV negative and positive cases (table 1). Although based on small numbers, warty and subtypes with basaloid features were observed more frequently among HPV positive cancers, reaching significance for the latter group only ($p=0.08$ and $p=0.010$, respectively). No differences in age or other clinical and pathological