The relationship of human papillomavirus positivity with tumor characteristics in an Irish penile cancer population

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ABSTRACT

Introduction: Penile cancer is a rare malignancy, with a European-wide annual incidence rate of 1/100 000 males. Approximately one-third of cases are attributable to human papillomavirus **(**HPV) infection. $p16^{INK4a}$ is a recognized surrogate marker for HPV infection in penile cancer. University Hospital Waterford (UHW) is the national referral center for penile cancer in Ireland. We report the prevalence of HPV infection and histological characteristics of an Irish penile cancer cohort using $p16^{INK4a}$ as a surrogate marker.
Methods: Patients who attended UHW for penile cancer surgery between June 2015 and November 2020 were entered into a prospectively maintained database. Clinical, histopathological, and outcome data were collected.
Results: Over the study period, 70 patients with a histological diagnosis of penile squamous cell carcinoma had staining for $p16^{INK4a}$, of whom 64% were positive. $p16^{INK4a}$ positive patients were significantly younger at diagnosis, with a mean age of 61±15 years compared to 68±12 ($p < 0.05$). Of note, 97% of tumors with high-risk histology were $p16^{INK4a}$ positive ($p<0.001$). $p16^{INK4a}$ positivity was more prevalent among higher-grade tumors ($p<0.02$). Interestingly, $p16^{INK4a}$ status was not associated with recurrence-free or overall survival.
Conclusions: Our data is representative of the Irish landscape in penile cancer over the last five years. Using $p16^{INK4a}$ staining, we demonstrate a high rate of HPV prevalence in penile cancer cases in our patient cohort, which is associated with prognostically worse tumor subtypes. This would suggest that HPV vaccination of adolescent boys is a useful public health intervention in preventing penile cancer in the Irish male population.

INTRODUCTION

Penile cancer is an uncommon malignancy with a reported annual incidence of 1 case per 100,000 males in the EU and USA.[1] There is noted to be substantial geographical variation in Europe of the incidence of penile cancer. The most recent Irish data reports an incidence of 1.45/100,000 cases per year.[2 3] There are two main recognised mechanisms for the development of penile cancer: the first resulting from chronic inflammation of the penis secondary to phimosis, recurrent balanitis and balanitis xerotica obliterans; and the second being human papillomavirus (HPV) infection.[1 4] Approximately one third of cases of penile cancer are attributed to HPV infection, with HPV 16 and 18 being the most common oncogenic subtypes isolated in resection specimens.[1 5]

$p16^{INK4a}$ is a tumour suppressor protein that inhibits kinases involved in the cell cycle.[6] It has been shown to be overexpressed in the pathogenesis of HPV infections; as such, $p16^{INK4a}$ protein expression is a recognised surrogate marker for HPV infection in penile cancer and its use in the histopathological reporting of penile cancer is recommended by the European Association of Urology (EAU).[1 6]

University Hospital Waterford is the national referral centre for penile cancer in Ireland. The aim of this study was to investigate the prevalence of HPV infection in an Irish penile cancer centre using $p16^{INK4a}$ as a surrogate marker. Furthermore, we aimed to compare histological characteristics and short-term outcomes between patients with p16-positive and -negative malignancies.

METHODS

Data collection

Data on all patients who attended University Hospital Waterford for penile cancer surgery between June 2015 to November 2020 was collated in a prospectively maintained database. Data points collected included demographic characteristics, surgical procedures undertaken and histopathological results including stage, grade and pathological subtype. Overall and recurrence-free survival was determined by clinic correspondence, imaging accessed on the National Imaging Management System (NIMIS) and histology reports from documented recurrent procedures. Immunohistochemical staining for $p16^{INK4a}$ was performed according to the manufacturer's protocol using a mouse monoclonal primary antibody $p16^{INK4a}$ Clone G175-405 (Zeta Corporation, Arcadia, CA, USA). Patients who did not have $p16^{INK4a}$ staining performed were excluded from the final analysis.

Statistical analysis

All data were tabulated using Microsoft Excel 2020 (Microsoft Corporation, Redmond, WA, USA) and Statistical Package for the Social Sciences (SPSS) software Version 24.0 (IBM SPSS Inc., Armonk, NY, USA. IBM Corp) was used for data analysis. Normally distributed continuous data were expressed as mean ± standard deviation (SD), while median (interquartile range) was used to describe the non-normally distributed continuous data. Categorical variables were presented as count and percent. Chi-square and one-way ANOVA tests were used to assess significance between categorical variables. Non-parametric data was

assessed using a Mann-Whitney U test or a Kruskall-Wallis test, depending on the number of variables involved in the analysis. Kaplan Meier Survival curves (log rank Mantel-Cox) and life tables analyses were used to compare recurrence-free survival and overall survival between the two cohorts at forty-eight months. A p-value of <0.05 was considered statistically significant.

RESULTS

81 patients underwent surgery for penile cancer in University Hospital Waterford between June 2015 and November 2020 for whom we had data. Eighty patients had a histological diagnosis of squamous cell carcinoma of the penis and 70 patients had immunohistochemical staining for $p16^{INK4a}$. Of the 70 patients who had staining for $p16^{INK4a}$, 64% (n=45) were positive. The median age at surgery was 65.14 years (IQR 51.44–75.62). Notably, $p16^{INK4a}$ positive patients were significantly younger at diagnosis compared to $p16^{INK4a}$ negative patients, with median ages of 60.6 and 72.4 years, respectively (p=0.024).

Relationship between p16 status and tumor characteristics

Sixty-eight patients were included in the final analysis investigating the impact of p16 status on tumour characteristics and outcomes. A specific breakdown is available in *Appendix 1*. We looked specifically at the relationship between p16 status and tumour histology. The differences in tumour characteristics between the p16-positive and negative subcohorts are tabulated in Table 1. Of note, p16 positivity was significantly more prevalent in patients with the histological subtypes known to confer higher risk, basaloid, warty basaloid and sarcomatoid (n=32/33, 97.%), compared to histological subtypes that carry a better prognosis (n=13/35, 37%) (p<0.001). The prevalence of p16 positivity increased incrementally from grade 1 (0%) to grade 2 (60%) to grade 3 (73%), and significantly more high grade tumours were p16-positive (p=0.013). There was no significant association between p16 status and tumour stage (p=0.631), the presence of lymphovascular space invasion (p=0.390), perineural invasion (p=0.489) and lymph node status (p=0.964).

Followup and survival outcomes

The median follow-up was 17.5 months (IQR 12.0–31.5). Eight patients (11.4%) had a recurrence detected during surveillance. Of these eight patients, 37.5% involved local recurrence at the penis, 37.5% were to regional lymph nodes and 25% were to pelvic lymph nodes. The median time to first recurrence was 12.00 months (IQR 7.0–29.5). There was no significant difference in either the recurrence rates between p16-positive and p16-negative patients (11.1% vs 13%, p=1.00), or the interval between surgery and recurrence (median 12.0 vs 12.0 months, p=1.0). Furthermore, Kaplan-Meier survival analysis demonstrated no significant difference in freedom from recurrence between p16+ve and p16-ve patients (p=0.918) (Figure 1). The number of patients at risk at each time point is illustrated in a lifetable analysis (Table 1).

The overall mortality rate during follow-up was 11.7% (n=8/68) and the median time from surgery to death was 14.5 months (IQR 5.75–17.75). There was no significant association between p16 status and all-cause mortality (p16 +ve 11.11% vs p16 -ve 13.0%,

p=1.00). Furthermore, Kaplan-Meier survival analysis demonstrated no significant difference in overall survival between p16-positive and p16-negative patients (p=0.839) (*Figure 2*).

DISCUSSION

HPV prevalence in an Irish penile cancer cohort

To our knowledge, this paper is the first to report on the relationship between $p16^{INK4a}$ positivity and penile cancer in an Irish population. $p16^{INK4a}$ is widely used as a surrogate marker for HPV positivity in many types of squamous cell carcinoma, namely cervical cancer.[6] Rates of HPV infection in penile cancer reportedly range from 33.3–56%.[1 7 8] However, a recent paper suggests HPV positivity in penile cancer is subject to geographical variability, reporting prevalence of HPV in invasive penile cancer to be 32.2% in Europe, 18.8% in North America, 36.5% in Latin America, 36.8% in Africa, 13.4% in Asia and 55.6% in Oceania.[9] In our population, 64.2% of cases were associated with HPV infection as determined by $p16^{INK4a}$ positivity. This is higher than the European average reported both by Alemany et al and the European Association of Urology (EAU).[1 2 9] Interestingly, our data suggests that patients with HPV positivity were significantly younger at initial presentation, which does not reflect the wider literature on penile cancer.[10] However, it has been reported in an Irish anal cancer population that HPV-positive patients were diagnosed significantly younger. [11]

The impact of p16 status on histological characteristics

Our data does not show a significant relationship between $p16^{INK4a}$ positivity and tumour stage, nodal status, LVSI or PNI. This is consistent with previous studies.[12] Interestingly, we did identify a significant association between $p16^{INK4a}$ positivity and tumour grade, whereby all $p16^{INK4a}$ positive patients had at least grade 2 tumours (p=0.013). Tumour grade has been shown to be an independent prognostic indictor for penile squamous cell carcinoma, with a higher grade tumour associated with a poorer prognosis.[13]

The impact of p16 status on survival outcomes

Current evidence suggests that HPV infection in penile cancer carries a better 5-year recurrence-free survival and overall survival than patients who are HPV negative.[12 14 15] HPV infection is also associated with improved loco-regional control of penile squamous cell carcinoma. [16] Given the rarity of penile cancer, these studies are limited by small sample size, as was our own paper. Considering this, it is interesting that in both our cohort and previously reported studies, p16 positivity was significantly associated with tumour subtypes which carry a poorer prognosis.[12] This was also seen in our follow-up data, which demonstrated that patients with p16 positivity had a higher recurrence free survival. However these patients had a higher all cause mortality Unfortunately these results did not reach statistical significance but this may be due to the small sample size of our cohort given the rarity of this disease.

HPV vaccination as a public health priority for cancer management

As previously mentioned, the most common HPV strains identified in penile cancer specimens are 16 and 18.[1] The HPV vaccine currently in use in Ireland is Gardasil 9 which is

a 9-valent vaccine which prevents against infection with HPV 16 and 18 among 7 other strains.[17] This vaccine has been offered to adolescent girls since 2010 and offered to adolescent boys from September 2019 as part of a national vaccination program in Ireland.[18] The high prevalence of $p16^{INK4a}$ positivity in our cohort of penile cancer patients likely reflects that of the general population given the wide range of referral sources to our centre. In addition, rates of penile cancer in Ireland have been shown to be increasing over time.[19] This demonstrates that HPV vaccination is a worthwhile public health measure in adolescent males, although in spite of a widespread recommendation to vaccinate adolescent boys international studies have suggested that such programs are not cost-effective and relied on vaccination of women with the aim of achieving herd immunity.[20]

Limitations

The rarity of this cancer and the ensuing small sample size is a significant limitation of this study. While certain patient details were documented prospectively, a moderate amount of the data analysed in this study was collected retrospectively and as such missing data was an intermittent challenge. Furthermore, the nature of UHW being a national referral centre meant outcome data was inconsistently documented, as patients were often followed-up by their local urology or oncology services. This meant that crucial data relating to cause of death was not available to us as there is no central patient record in Ireland.

CONCLUSIONS

Penile squamous cell carcinoma is a rare cancer worldwide. Rates of penile cancer are increasing in Ireland. Our data is representative of the Irish landscape in penile cancer over the last five years, given Ireland's homogenous population and the wide range of referral sources for our cohort. Using $p16^{INK4a}$ staining as surrogate marker, our data demonstrates a high HPV prevalence in penile cancer cases in our patient cohort. Furthermore, $p16^{INK4a}$ positivity was significantly associated with certain tumour subtypes known to carry a poorer prognosis. This would suggest that HPV vaccination of adolescent boys is a useful public health intervention in the prevention of penile cancer in the Irish male population.

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FIGURES AND TABLES

Figure 1. Kaplan-Meier survival analysis comparing recurrence-free survival at 48 months of followup between patients with p16+ve and p16-ve tumours. No significance difference noted on log rank (Mantel-Cox) analysis.

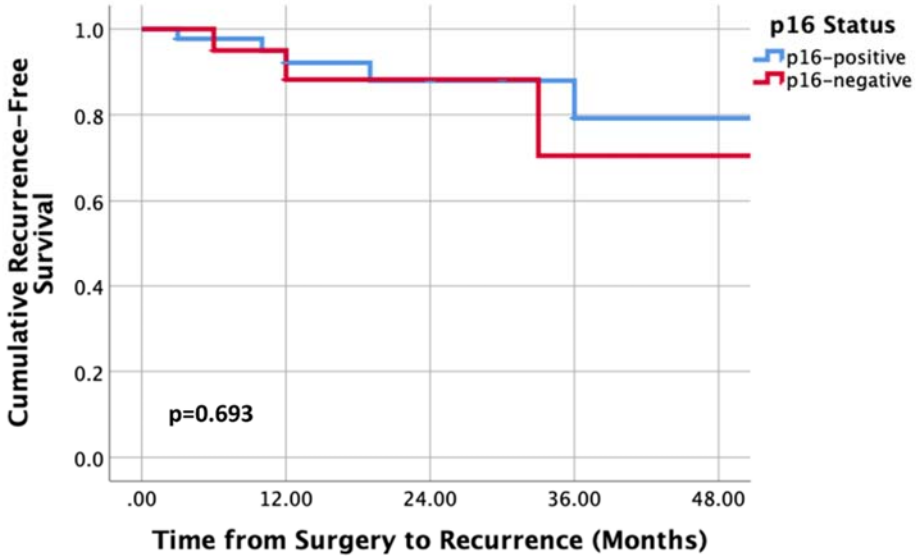

Figure 2. Kaplan-Meier survival analysis comparing overall survival from all-cause mortality at 48 months of followup between patients with p16+ve and p16-ve tumours. No significance difference noted on log rank (Mantel-Cox) analysis.

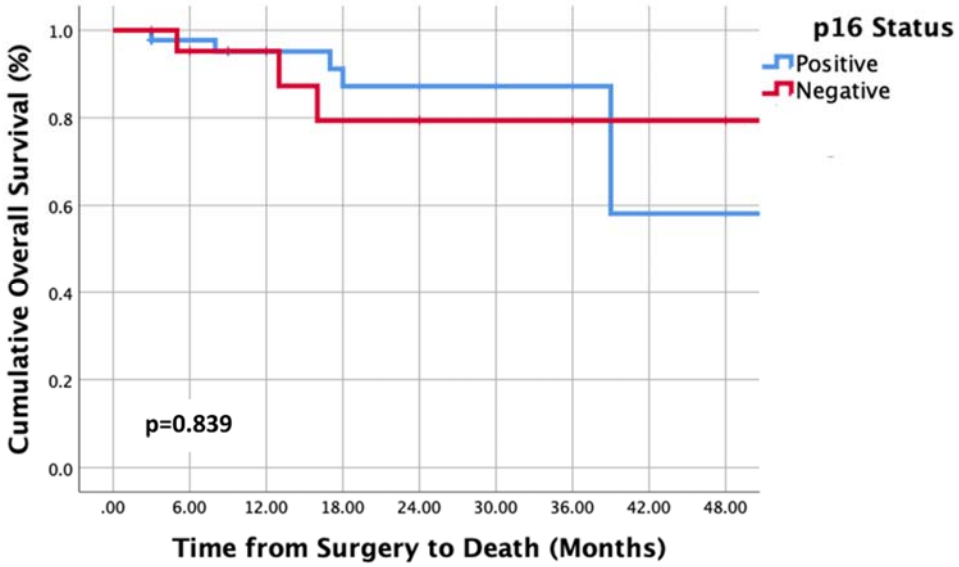

Table 1. Tabulation of p16+ve prevalence across tumor characteristics commonly associated with poorer prognosis			
Tumour characteristics	**p16 +ve**	**p16 -ve**	**p**
Histology (n=68) High-risk (basaloid, Warty-Basaloid, sarcomatoid)	71.1% (32/45)	4.3% (1/23)	<0.001*
Tumor stage (n=68) Early-stage (T1a) Late-stage (T3–4)	 17.6% (6/34) 20% (9/45)	 30% (6/20) 13% (3/23)	 0.292 0.477
Tumor grade High-grade (G2–3)	 100% (34/34)	 78.9% (15/19)	 0.013**
Lymphovascular space invasion LVSI present	 36.36% (12/33)	 25% (5/20)	 0.390
Perineural invasion Present	 39.39% (13/33)	 30% (6/20)	 0.489
Lymph node status Positive nodes	 22.22% (10/45)	 21.7% (5/23)	 0.964

Categorical variables were compared using Chi-square analysis, except for tumor grade where Fisher's exact test (2-sided) was used. LVSI: lymphovascular space invasion.