Title: Penile cancer and the HPV attributable fraction in Scotland; a retrospective cohort study.

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Running Title: Implications of HPV infection in penile cancer

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Keywords: HPV, Penile Cancer, Prevalence, Prognosis

Word count = 2219

This is a peer-reviewed, accepted author manuscript of the following research article: Cuschieri, K., Pan, J., O Donnell, M., Kirkwood, K., Kavanagh, K., Pollock, K. G., Bhatia, R., Graham, S. V., & Wakeham, K. (2021). Penile cancer and the HPV attributable fraction in Scotland; A retrospective cohort study. *Journal of Clinical Virology*, *134*, [104717]. https://doi.org/10.1016/j.jcv.2020.104717

ABSTRACT

Background: Penile cancer (PeC) is a highly morbid disease which is rising in certain settings including Scotland. A component of PeC is associated with Human Papillomavirus (HPV) although its influence on clinical outcomes is debatable as is whether the fraction attributable to HPV is increasing.

Methods: A total of 122 archived tissue samples derived from patients diagnosed with PeC between 2006-2015 were collated and tested for HPV DNA using molecular PCR. HPV positivity was determined for the overall population and by calendar year of diagnosis to determine any temporal trends. The influence of age, deprivation, smoking, tumour stage and tumour grade on likelihood of HPV positivity was determined by logistic regression. In addition, the influence of HPV status and the other clinical and demographics variables on all-cause death and death from PeC was assessed.

Results: HPV was detected in 43% (95% CI: 34-52) of penile cancers and the majority of infections were HPV 16 . The HPV component of PeC did not increase over the time period (p for linear trend – 0.226). No demographic or clinical variables were associated with HPV positivity neither was HPV status associated with improved all-cause or cancer-specific survival during the follow up period.

Conclusion: The rise in PeC in Scotland may not be attributable to a rise in HPV-associated cancer; this is consistent with oropharyngeal cancer (OPC) in the UK where there is an increase in both HPV positive and negative cancer. This work calls for a larger multi centre study to enable further detailed investigation into the implications of HPV infection in PeC.

INTRODUCTION

Penile cancer (PeC) is a highly morbid condition and evidence suggests its incidence is increasing, including in Scotland. National cancer registry data show an increase in European Age Standardised Rate (EASR per 100,000) from 1.5 (1.0-2.1) in 1993 to 3.4 (2.7-4.2) in 2017 (1). This increase is consistent with other data from Scotland that indicate a rise in non-cervical HPV associated cancer (2,3). While PeC may be a relatively rare cancer in Europe and North America it accounts for up to 10% of male cancer in resource-limited settings in South America, Africa and Asia (4).

The role and influence of HPV on disease progression and other clinical outcomes in PeC is less clear than for other HPV associated neoplasms (such as oropharynx); this is partly due to its comparative rarity and arguably a paucity of research. There is some evidence to suggest that the mutational landscape of HPV associated versus HPV negative PeC may be different and that this may have implications for the trajectory of disease (5,6). Furthermore, two recent meta-analyses on the burden of HPV associated penile cancer and the clinical implications of viral positivity have been welcome (7,8). Key findings from this work included the observation that while 51% of penile cancers were HPV positive overall (in an assessment of 4199 cases), this prevalence varied considerably according to geography, with 41.9% (22.6-62.5%) positive in Asia to 87.5% (75.6-95.8) in Africa. Primary treatment tends to be surgical, from local excision, glansectomy, partial penectomy and total penectomy depending on the stage of disease at presentation. Sentinal node biopsy is initially carried out in high risk disease where nodes are clinically or radiologically univolved. This is followed by regional lymph node dissection, where positive. Chemotherapy and radiotherapy may be used in the adjuvant curative setting or for palliation (4).

Additionally, with respect to HPV status and prognosis from PeC, Sand and colleagues (2018) found that HPV driven cancers were associated with favourable clinical outcome. Other

described risk factors for PeC relate to genital hygiene, chronic inflammation, phimiosis, HIV, smoking and genital warts (9,10). Circumcision appears protective due to the association with reduction in the rates of penile inflammatory disease, as well as improved hygiene (11).

As elegant as these analyses were, the authors were not able to stratify HPV status according to date of diagnosis or identify temporal trends to determine whether the HPV component of penile cancer has risen over time. Given the morbidity of this cancer and the potential protective effect of HPV vaccine on future generations of men, understanding local epidemiology is of importance. This study aimed to assess the HPV status of a population based cohort of penile cancer over time and to determine whether HPV status linked with survival.

MATERIALS AND METHODS

Governance and sample collection strategy

Cases of pathologically confirmed PeC diagnosed in the South East of Scotland Cancer Network between 2006 and 2015 (n=122) were identified from pathological records. The South East Scotland Cancer Network covers a population of 1.4 million across four health boards (Borders, Dumfries & Galloway, Fife and Lothian) and represents around 20% of all PeC diagnosed in Scotland. Individual management plans were formed following discussion at the regional Uro-Oncology Multidisciplinary meeting. diagnosis and treatment is in line with the guidelines published by the European Association of Urology (4), which is endorsed by the British Association of Urological Surgeons. All individuals accessing health care in Scotland are assigned a unique 10-digit number, which allows linkage of clinical, social and laboratory data. Sociodemographic and clinical data were extracted from digital clinical records. The variables collected for this study were - Date of diagnosis - taken as date of initial diagnostic biopsy sample collection, age at diagnosis, cancer stage using 8th edition

TNM Classification of Malignant Tumors (TNM) (12), history of ever smoking and date of death. In addition, area-based socioeconomic status was obtained - via the Scottish Index of Multiple Deprivation (SIMD) where 1 and 5 are the most and least deprived respectively.

The pathological material was reviewed by two experienced consultant Uro-pathologists with cases referring to individuals as opposed to individual episodes. Histological sub-type of cancer is reported. The relevant formalin fixed paraffin embedded block was then retrieved for downstream HPV testing. Use of samples for the present work was facilitated by the South East of Scotland bioresource (Application SR621). Data on patient outcomes were made available via the Scottish safe haven after application to the public benefit and privacy panel for health and social care.

HPV DNA testing and assessment

A 10 micron section of the formalin fixed paraffin embedded block was used for nucleic acid extraction using the reagents within the Qiagen DNA mini kit and a method optimised for the molecular detection of HPV, this includes an extended incubation with proteinase K for a minimum of 12 hours as per Steinau et al (13). HPV testing was performed using three separate PCR based HPV assays, the Optiplex HPV Genotyping test (Diamex, Heidelberg, Germany), the Venus HPV Test (LiferRiver, Shanghai, China) and the Harmonia HPV Test (LifeRiver, Shanghai, China). All tests detect the range of established high-risk HPV types as defined by the International Agency of Research on Cancer; Optiplex and Harmonia also detect common low-risk types including HPV 6 and 11. This approach was followed given the age range of the FFPE blocks and the comparative lack of validation data on appropriate HPV tests for annotation of PeCs. All tests contained an endogenous human cellular amplification control to minimise false negatives. A combined “final” result was generated if 2/3 tests were concordant. A test was considered invalid if it tested HPV negative and also negative

for the endogenous control. HPV status was stratified as HPV positive "any" – which could have included low-risk types; high-risk HPV positive, high-risk HPV positive for HPV 16 and high-risk positive for HPV 16 and/or 18.

Final study set

Of the 122 cases of squamous penile cancer, 6 were excluded from further analysis; 1 was excluded as it was not a confirmed invasive cancer, 2 were excluded due to missing or unclear clinical/follow up information and 3 were excluded on the basis of invalid HPV test results (ie HPV negative and endogenous control negative). This left a final evaluable sample of 116.

Analysis of HPV status according to temporal, clinical and demographic variables

HPV status (considered as "any" HPV positivity) was stratified by age at resection, SIMD, smoking status, cancer staging with the TNM 8th edition of the classification of malignant tumours and tumour grade. Those whose lymph node status could not be assessed (Nx) were classified according to their T stage. The proportion of cases "HPV positive according to year of resection" was calculated and plotted with 95% confidence intervals. A linear test of trend for HPV "any" status and 16/18 status over resection year was also performed. Odds ratios (ORs) to be HPV positive ("any") were calculated for the variables described; presented as a univariate and adjusted analysis. A sensitivity analysis of all cause survival was performed to exclude 3 TNM 8 Nx patients to avoid any potential misclassification.

Influence of HPV status on Survival

Individuals were followed up until time of death or date leaving Scotland. Kaplan Meier curves were produced split by HPV ("any") status with all cause death and death associated with PeC presented separately. Hazard ratios were assessed using cox proportional hazards model and unadjusted and adjusted results presented with adjustments made for age, deprivation, smoking, and TNM 8 stage and tumour grade.

RESULTS

Morphology and proportion of HPV positive PeC in Scotland overall and over time

The three most common histological subtypes in the cohort were usual (70%) warty (11%) and basoloid (10%), the remaining fraction was composed of papillary, verrucous, mixed, sarcomatoid and hyperplastic types. When considering the three most common subtypes, 40% of the usual type, 34% of the warty type and 100% of the basoloid type were HPV positive. Table 1 and Table 2 detail the overall demographics of the population and the proportion of HPV positive cases, respectively. Overall a total of 50/116 cases were HPV positive (43%, 95% CI: 34-52) with 49/116 positive for at least 1 high risk type (42%, 95% CI: 34-51%). HPV type 16 dominated as 42/116 (36%; 95% CI: 28-45%) cases tested positive for this type as a single or within a mixed infection. Figure 1 shows the proportion of HPV positive cases stratified by year. We did not observe any clear changes in HPV proportion over time; the linear trend tests for "any" HPV positive or 16/18 positive over time were p=0.226 and p=0.674 respectively.

Influence of demographic and clinical variables on HPV status

In the unadjusted analysis, HPV positivity was not associated with age at resection, smoking status, age, TNM8 stage, grade or deprivation (Table 3). Similar observations were made for the adjusted analysis.

Influence of HPV status on survival

Follow-up information was available from patients for an average of 4.84 years (IQR 2.98-7.19 years). Over the time period 40 individuals died, 22 of whom were confirmed as having died from PeC. Although estimated as a hazard which reduced the outcome, HPV (positive) status was not significantly associated with improved survival, both for all-cause death and death from PeC with adjusted hazard ratios of 0.57 (95% CI: 0.27-1.1.7) and 0.43 (95%CI: 0.13-1.41) respectively - see Figure 2 and Table 4 (all-cause death) and Figure 3 and Table 5 (PeC death). Sensitivity analysis, excluding the Nx patients, also found similar results. Factors that did significantly influence all cause death were age when age 76+ vs. <=55 years old age groups were compared (with a hazard ratio of 19.98 (95%CI: 5.49-72.68_ in the adjusted analysis respectively) and TNM8 stage, when comparing stage 1 to 4 with an adjusted HR of 21.24 (95% CI: 5.38-83.90). Factors that influenced death from PeC were, again age adjusted HR = 60.37 (95% CI: 6.07-600.35) and stage (adjusted HR = 281.55 (95%CI 22.24-3,564).

DISCUSSION

HPV was associated with just under half of this Scottish cohort of penile cancer cases, with the majority of infections being high-risk HPV types. The proportion of HPV associated PeC does not appear to have increased over time and therefore may not account for the overall rise in this disease in Scotland and indeed in other contexts, reasons for an increase in PeC are still unclear. While it is feasible that changed sexual and hygiene practices may exert an influence, we did not specifically collate this data and future studies which address this would be welcome. While there have been recent reports on HPV prevalence in PeC, there

are no large series' to our knowledge where HPV positive status has been presented according to year of diagnosis in a time frame that spans more than 5 years.

These data are consistent with those described by Schache *et al* (2016) who assessed HPV presence in a UK based multi-centre study of over 1000 oropharyngeal cancers over a period of 10 years. In the work the authors observed that while HPV associated OPC was increasing, so was the non-viral associated cancer component and that the proportion of HPV positive disease had not changed over time (14).

Taken as a cross sectional assessment, overall positivity in the Scottish cohort was in line with European average taken from the meta-analysis (7) which at 50.3 (39.8-60.9) compared to 45% (95%CI 36-54) reported in the present work. HPV 16 dominated the HPV positive component which, while expected, is nevertheless an encouraging observation when thinking about the component that will be vaccine preventable (15).

No variables were independently associated with HPV positive status in this population. The direction of the effect for HPV positivity showed improved survival for all-cause death or from death specifically from PeC but this was not statistically significant. This lack of significant effect is at variance with the meta-analysis (8) but is likely a limitation of our small sample size, which was curtailed by the rarity of the disease. In addition, we did not perform p16INK4a testing which may be more indicative of HPV driven disease in the penile context as observed by its higher prognostic significance; in the 20 studies reviewed by Sand *et al* 2019 (7) a pooled hazard ratio for HPV positive PeC was 0.61 (95% CI 0.39-0.98) vs 0.45 for p16INK4a positive PeC (95% CI 0.30-0.69).

The strengths of this study are that it represented a population based cohort, HPV testing was performed centrally and annotation/adjustment for key clinical and demographic variables was made. There are very few data in the UK on HPV prevalence in PeC so, as described, this piece provides local information on the component that may be vaccine

preventable. The weaknesses are that the cohort was relatively small, we had no data on patient behaviours and practices or data on HPV transcriptional activity. Given the comparative rarity of PeC we would suggest that a UK multi-centre approach taking advantage of Supra-regional Penile cancer networks would be extremely valuable for the further interrogation of this disease in terms of its risk-factors, epidemiology, underlying molecular biology - and crucially, natural history including at the pre-invasive stage (16) . We would argue this is apposite given the increase in PeC and challenges of managing this morbid disease which can be intractable to conventional management and treatment strategies and predispose to future HPV associated malignancies (17).

ACKNOWLEDGMENTS

We would like to thank all members of the Scottish HPV Reference Laboratory and the HPV Research group for support with sample processing and testing. Thanks are also due to NRS Lothian Bioresource (formerly SAHSC Bioresource) for support with sample capture and governance.

Conflicts: K Cuschieri, R Bhatia (non personal) K Cuschieri & R Bhatia's institution has received research funding or gratis consumables to support research from the following commercial entities in the last 3 years: Cepheid, Genomica, LifeRiver, Euroimmun, GeneFirst, SelfScreen, Qiagen, Hiantis, Seegene and Hologic. K Pollock has received consultancy fees from MSD, UK. No other authors report a conflict of interest in relation to this work.

REFERENCES

1 https://www.isdscotland.org/Health-Topics/Cancer/Cancer-Statistics/Male-Genital-Organs/

2: Wakeham K, Kavanagh K. The burden of HPV-associated anogenital cancers. Curr Oncol Rep. 2014 Sep;16(9):402.

3: Deshmukh AA, Suk R, Shiels MS, Sonawane K, Nyitray AG, Liu Y, Gaisa MM, Palefsky JM, Sigel K. Recent trends in squamous cell carcinoma of the anus incidence and mortality in the United States, 2001-2015. J Natl Cancer Inst. 2019 Nov 19. pii: djz219.

4. European Association of Urology oncology guidelines on Penile Cancer diagnosis and management, 2018. Access on 3rd December 2020. https://uroweb.org/guideline/penile-cancer/

5: Feber A, Worth DC, Chakravarthy A, de Winter P, Shah K, Arya M, Saqib M, Nigam R, Malone PR, Tan WS, Rodney S, Freeman A, Jameson C, Wilson GA, Powles T, Beck S, Fenton T, Sharp TV, Muneer A, Kelly JD. CSN1 Somatic Mutations in Penile Squamous Cell Carcinoma. Cancer Res. 2016 Aug 15;76(16):4720-4727.

6: Eich ML, Del Carmen Rodriguez Pena M, Schwartz L, Granada CP, Rais-Bahrami S, Giannico G, Amador BM, Matoso A, Gordetsky J. Morphology, p16, HPV, and outcomes in squamous cell carcinoma of the penis: a multi-institutional study. Hum Pathol. 2019 Nov 4. pii: S0046-8177(19)30192-3.

7: Olesen TB, Sand FL, Rasmussen CL, Albieri V, Toft BG, Norrild B, Munk C, Kjær SK. Prevalence of human papillomavirus DNA and p16(INK4a) in penile cancer and penile

intraepithelial neoplasia: a systematic review and meta-analysis. Lancet Oncol. 2019 Jan;20(1):145-158.

8: Sand FL, Rasmussen CL, Frederiksen MH, Andersen KK, Kjaer SK. Prognostic Significance of HPV and p16 Status in Men Diagnosed with Penile Cancer: A Systematic Review and Meta-analysis. Cancer Epidemiol Biomarkers Prev. 2018 Oct;27(10):1123-1132.

9: Emmanuel A, Nettleton J, Watkin N, Berney DM. The molecular pathogenesis of penile carcinoma-current developments and understanding. Virchows Arch. 2019 Oct;475(4):397-405. doi: 10.1007/s00428-019-02607-8. Epub 2019 Jun 26. Review. PubMed PMID: 31243533.

10: Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, Carter JJ, Porter PL, Galloway DA, McDougall JK, Krieger JN. Penile cancer: importance of circumcision, human papillomavirus and smoking in in situ and invasive disease. Int J Cancer. 2005;116:606–16.

11: Maden C, Sherman KJ, Beckmann AM, Hislop TG, Teh CZ, Ashley RL, Daling JR. History of circumcision, medical conditions, and sexual activity and risk of penile cancer. J Natl Cancer Inst. 1993;85:19–24.

12: Hölters S, Khalmurzaev O, Pryalukhin A, Loertzer P, Janssen M, Heinzelbecker J, Ueberdiek S, Pfuhl T, Smola S, Agaimy A, Geppert C, Loertzer H, Krah X, Wunderlich H, Wagenpfeil S, Bohle RM, Stöckle M, Matveev V, Hartmann A, Junker K. Challenging the prognostic impact of the new WHO and TNM classifications with special emphasis on HPV status in penile carcinoma. Virchows Arch. 2019 Aug;475(2):211-221.

13. Steinau M, Patel SS, Unger ER.J Efficient DNA extraction for HPV genotyping in formalin-fixed, paraffin-embedded tissues. Mol Diagn. 2011 Jul;13(4):377-81.

14: Schache AG, Powell NG, Cuschieri KS, Robinson M, Leary S, Mehanna H, Rapozo D, Long A, Cubie H, Junor E, Monaghan H, Harrington KJ, Nutting CM, Schick U, Lau AS, Upile N, Sheard J, Brougham K, West CM, Oguejiofor K, Thomas S, Ness AR, Pring M, Thomas GJ, King EV, McCance DJ, James JA, Moran M, Sloan P, Shaw RJ, Evans M, Jones TM. HPV-Related Oropharynx Cancer in the United Kingdom: An Evolution in the Understanding of Disease Etiology. Cancer Res. 2016 Nov 15;76(22):6598-6606..

15: de Sanjosé S, Serrano B, Tous S, Alejo M, Lloveras B, Quirós B, Clavero O, Vidal A, Ferrándiz-Pulido C, Pavón MÁ, Holzinger D, Halec G, Tommasino M, Quint W, Pawlita M, Muñoz N, Bosch FX, Alemany L; RIS HPV TT, VVAP and Head and Neck study groups . Burden of Human Papillomavirus (HPV)-Related Cancers Attributable to HPVs 6/11/16/18/31/33/45/52 and 58. JNCI Cancer Spectr. 2019 Jan 7;2(4):pky045.

16: Gilbert DC, Wakeham K, Langley RE, Vale CL. Increased risk of second cancers at sites associated with HPV after a prior HPV-associated malignancy, a systematic review and meta-analysis. Br J Cancer. 2019 Jan;120(2):256-268

17. Lieblong BJ, Montgomery BEE, Su LJ, Nakagawa M Natural history of human papillomavirus and vaccinations in men: A literature review. Health Sci Rep. 2019 Mar 12;2(5):e118.

Variable	Level	Number
HPV status	negative	66
	positive	50
Age at resection (n;%)	<=55	29
	56-65	31
	66-75	25
	76+	31
SIMD	1: most deprived	20
	2	25
	3	29
	4	22
	5: least deprived	20
Smoking status	Non Smoker	28
	Smoker	15
	Ex smoker	22
	Unknown	51
TNM8 stage	I	27
	IIA	31
	IIB	12
	IIIA	21
	IIIB	9
	IV	16
Grade	G1	19
	G2	63
	G3	34

Table 1 –Demographic and clinical variables related to final analysis set (n=116). SIMD = Scottish index of multiple deprivation. 3 individuals are removed from the full analysis due to missing grade (n=1) and inability to map to TNM8 stage (n=2) (T stage was PTX or PTA and N stage Nx).

HPV type	Number Positive	Proportion positive(/116)	95% CI
HPV (Any)	50	43.1%	(34.5, 52.2)%
HR-HPV[1]	47	40.5%	(32.0, 49.6)%
HPV 16 alone	36	31.0%	(23.3, 39.9)%
HPV 16[2]	40	34.5%	(26.5, 43.5)%
HPV16 and/or 18	43	37.1%	(28.8, 46.1)%

Table 2: HPV positivity in 116 penile cancer cases diagnosed in the South East of Scotland between 2006 and 2015
Percentage positive are presented with 95% confidence intervals.
[1] – HPV positive for HPV 16,18,31,33,35,39,45,51,52,56,58,59,68
[2] – Any appearance (as single or part of mixed infection)

Variable	Level	N	% (/116)	N HPV+	%HPV+ (/N)	Unadjusted OR (95% CIs)	p value	Adjusted OR (95% CIs)	p value
Age at resection (n;%)	<=55	29	25	12	41.4	1		1	
	56-65	31	26.7	13	41.9	1.02 (0.37-2.86)	0.97	1.24 (0.39-3.87)	0.72
	66-75	25	21.6	11	44	1.11 (0.38-3.28)	0.85	0.64 (0.18-2.2)	0.47
	76+	31	26.7	14	45.2	1.17 (0.42-3.25)	0.77	1.09 (0.34-3.5)	0.89
Age at resection (median; IQR)		65	55.75-76	65.6	56-76				
SIMD	1: most deprived	20	17.2	7	35	1		1	
	2	25	21.6	13	52	2.01 (0.6-6.74)	0.26	2.05 (0.52-8.05)	0.3
	3	29	25	11	37.9	1.13 (0.35-3.72)	0.83	0.72 (0.18-2.85)	0.64
	4	22	19	9	40.9	1.29 (0.37-4.5)	0.69	0.92 (0.23-3.73)	0.91
	5: least deprived	20	17.2	10	50	1.86 (0.52-6.61)	0.34	2.08 (0.49-8.87)	0.32
smoke status	Non Smoker	28	24.1	11	39.3	1		1	
	Smoker	15	12.9	6	40	1.03 (0.29-3.71)	0.96	0.85 (0.19-3.83)	0.83
	Ex smoker	22	19	8	36.4	0.88 (0.28-2.8)	0.83	0.74 (0.2-2.71)	0.65
	Unknown	51	44	25	49	1.49 (0.58-3.79)	0.41	1.93 (0.65-5.73)	0.24
TNM8 stage	I	27	23.3	12	44.4	1		1	
	IIA	31	26.7	8	25.8	0.43 (0.14-1.31)	0.14	0.42 (0.12-1.46)	0.17
	IIB	12	10.3	4	33.3	0.63 (0.15-2.59)	0.52	0.5 (0.09-2.71)	0.42
	IIIA	21	18.1	14	66.7	2.5 (0.77-8.16)	0.13	3.24 (0.86-12.22)	0.08
	IIIB	9	7.8	5	55.6	1.56 (0.34-7.13)	0.56	1.13 (0.18-7.22)	0.9
	IV	16	13.8	7	43.8	0.97 (0.28-3.38)	0.96	1.08 (0.25-4.57)	0.92
grade	G1	19	16.4	9	47.4	1		1	
	G2	63	54.3	28	44.4	0.89 (0.32-2.49)	0.82	0.87 (0.25-3.01)	0.83
	G3	34	29.3	13	38.2	0.69 (0.22-2.14)	0.52	0.62 (0.14-2.77)	0.53

Table 3:. HPV status according to demographic and clinical variables

Var	Level	N (Total=116)	N died (all cause)(Total=40)	person years	rate per 100 person years	univariate HR	p value	Adjusted HR	p value
HPV	negative	66	26	369.4	7.04 (4.60-10.31)	1		1	
	positive	50	14	252.6	5.54 (3.03-9.30)	0.77 (0.40-1.48)	0.4328	0.57 (0.27-1.17)	0.1231
Age at resection (n;%)	<=55	29	6	193.2	3.11 (1.14-6.76)	1		1	
	56-65	31	8	190.0	4.21 (1.82-8.29)	1.36 (0.47-3.92)	0.5683	7.22 (1.83-28.43)	0.0047
	66-75	25	8	138.8	5.76 (2.49-11.36)	1.75 (0.61-5.05)	0.3014	4.66 (1.26-17.26)	0.0214
	76+	31	18	100.0	17.99 (10.66-28.44)	4.60 (1.80-11.77)	0.0015	19.98 (5.49-72.68)	0.0000
SIMD	1: most deprived	20	7	128.5	5.45 (2.19-11.22)	1		1	
	2	25	11	96.0	11.46 (5.72-20.50)	1.76 (0.68-4.59)	0.2447	1.06 (0.34-3.33)	0.9209
	3	29	9	150.1	6.00 (2.74-11.38)	1.02 (0.38-2.74)	0.9748	0.67 (0.21-2.12)	0.4957
	4	22	7	119.3	5.87 (2.36-12.09)	1.01 (0.35-2.88)	0.9886	1.13 (0.35-3.65)	0.8424
	5: least deprived	20	6	128.1	4.68 (1.72-10.19)	0.90 (0.30-2.69)	0.8556	0.74 (0.19-2.91)	0.6681
smoke status	Non Smoker	28	9	188.4	4.78 (2.18-9.07)	1		1	
	Smoker	15	6	73.1	8.21 (3.01-17.87)	1.48 (0.52-4.18)	0.4588	3.09 (0.96-9.97)	0.0588
	Ex smoker	22	7	115.6	6.06 (2.43-12.48)	1.17 (0.43-3.13)	0.7618	1.25 (0.42-3.71)	0.6914
	Unknown	51	18	245.0	7.35 (4.35-11.61)	1.36 (0.61-3.05)	0.4495	2.36 (0.88-6.32)	0.0865
TNM8 stage	I	27	7	175.1	4.00 (1.61-8.24)	1		1	
	IIA	31	6	192.7	3.11 (1.14-6.78)	0.78 (0.26-2.31)	0.6477	0.76 (0.24-2.43)	0.6483
	IIB	12	7	51.6	13.56 (5.45-27.94)	3.07 (1.08-8.78)	0.0360	8.31 (2.06-33.45)	0.0029
	IIIA	21	5	124.0	4.03 (1.31-9.41)	1.01 (0.32-3.19)	0.9824	0.96 (0.28-3.35)	0.9505
	IIIB	9	3	46.1	6.50 (1.34-19.00)	1.55 (0.40-6.00)	0.5263	2.91 (0.59-14.41)	0.1917
	IV	16	12	32.6	36.85 (19.04-64.36)	6.96 (2.67-18.18)	0.0001	21.24 (5.38-83.90)	0.0000
grade	G1	19	5	114.1	4.38 (1.42-10.23)	1		1	
	G2	63	22	370.3	5.94 (3.72-9.00)	1.36 (0.52-3.60)	0.5319	1.52 (0.47-4.94)	0.4878
	G3	34	13	137.7	9.44 (5.03-16.15)	1.86 (0.66-5.23)	0.2409	0.56 (0.13-2.43)	0.4349

Table 4: Survival (all cause death) stratified by HPV status, demographic variables and clinical variables. N =116 patients. Average follow up 4.84 years IQR 2.98-7.19 years

Var	Level	N (Total=116)	PeC death rate per 100 person years (95% CIs)	Unadjusted HR (95% CIs)	p value	Adjusted HR** (95% CIs)	p value
HPV	negative	66	3.25 (1.68-5.67)				
	positive	50	3.56 (1.63-6.76)	1.01 (0.42-2.39)	0.987	0.43 (0.13-1.41)	0.163
Age at resection (n;%)	<=55	29	1.55 (0.32-4.54)	1.00 (0.00-0.00)		1.00 (0.00-0.00)	
	56-65	31	2.10 (0.57-5.39)	1.30 (0.29-5.83)	0.728	35.45 (2.84-442.28)	0.006
	66-75	25	3.60 (1.17-8.41)	2.00 (0.48-8.37)	0.343	17.27 (1.86-160.51)	0.012
	76+	31	9.00 (4.11-17.08)	3.47 (0.94-12.87)	0.062	60.37 (6.07-600.35)	0.000
SIMD	1: most deprived	20	1.56 (0.19-5.62)	1.00 (0.00-0.00)		1.00 (0.00-0.00)	
	2	25	7.29 (2.93-15.02)	3.20 (0.66-15.44)	0.147	3.03 (0.32-28.34)	0.330
	3	29	3.33 (1.08-7.77)	1.76 (0.34-9.10)	0.497	1.58 (0.20-12.66)	0.668
	4	22	2.51 (0.52-7.35)	1.38 (0.23-8.27)	0.723	1.04 (0.09-11.85)	0.976
	5: least deprived	20	3.12 (0.85-7.99)	2.17 (0.40-11.82)	0.372	1.53 (0.13-18.54)	0.738
smoke status	Non Smoker	28	2.12 (0.58-5.44)	1.00 (0.00-0.00)		1.00 (0.00-0.00)	
	Smoker	15	2.74 (0.33-9.89)	1.00 (0.18-5.46)	1.000	2.02 (0.26-15.59)	0.501
	Ex smoker	22	4.33 (1.40-10.09)	1.73 (0.46-6.43)	0.416	2.49 (0.52-11.97)	0.253
	Unknown	51	4.08 (1.96-7.51)	1.55 (0.49-4.95)	0.459	4.76 (1.02-22.09)	0.047
TNM8 stage	I	27	0.57 (0.01-3.18)	1.00 (0.00-0.00)		1.00 (0.00-0.00)	
	IIA	31	0.52 (0.01-2.89)	0.89 (0.06-14.27)	0.936	0.94 (0.06-15.36)	0.967
	IIB	12	5.81 (1.20-16.99)	8.28 (0.86-79.72)	0.067	29.40 (1.87-461.37)	0.016
	IIIA	21	1.61 (0.20-5.83)	2.66 (0.24-29.30)	0.425	1.80 (0.14-23.12)	0.652
	IIIB	9	6.50 (1.34-19.00)	10.16 (1.06-97.71)	0.045	16.46 (1.02-266.05)	0.049
	IV	16	33.78 (16.86-60.44)	31.80 (4.08-248.04)	0.001	281.55 (22.24-3,564.14)	0.000
grade	G1	19	0.88 (0.02-4.88)	1.00 (0.00-0.00)		1.00 (0.00-0.00)	
	G2	63	3.78 (2.07-6.34)	4.40 (0.58-33.42)	0.153	3.41 (0.30-38.49)	0.321
	G3	34	4.36 (1.60-9.49)	3.81 (0.46-31.66)	0.216	0.17 (0.01-2.55)	0.202

Table 5: Survival (death from penile cancer) stratified by HPV status, demographic variables and clinical variables. N =116 patients. 22 deaths due to penile cancer over 622.1 person years. Number of penile cancer related deaths stratified by variable cannot be presented due to information governance disclosure control.

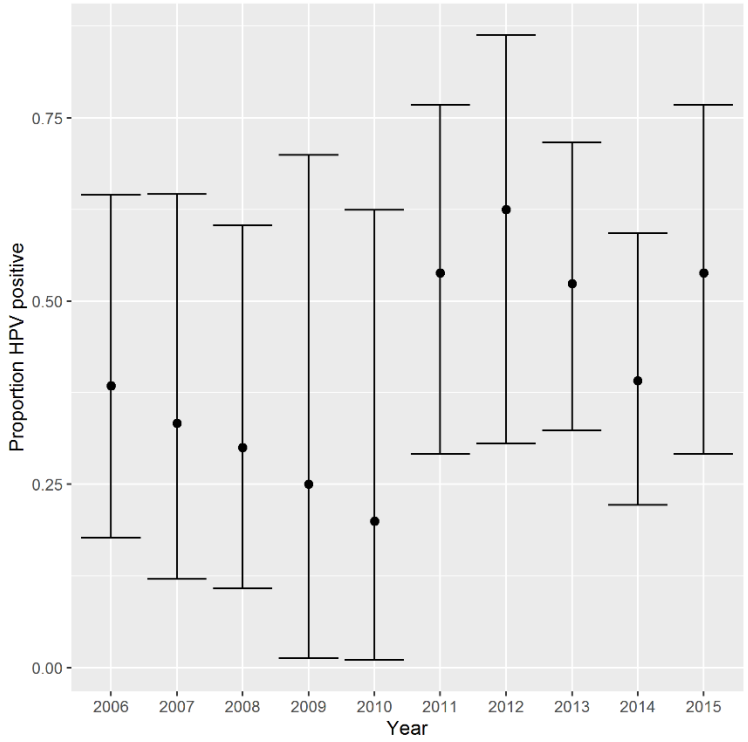
Proportion HPV positive
0.75
0.50
0.25
0.00
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Year

Figure 1

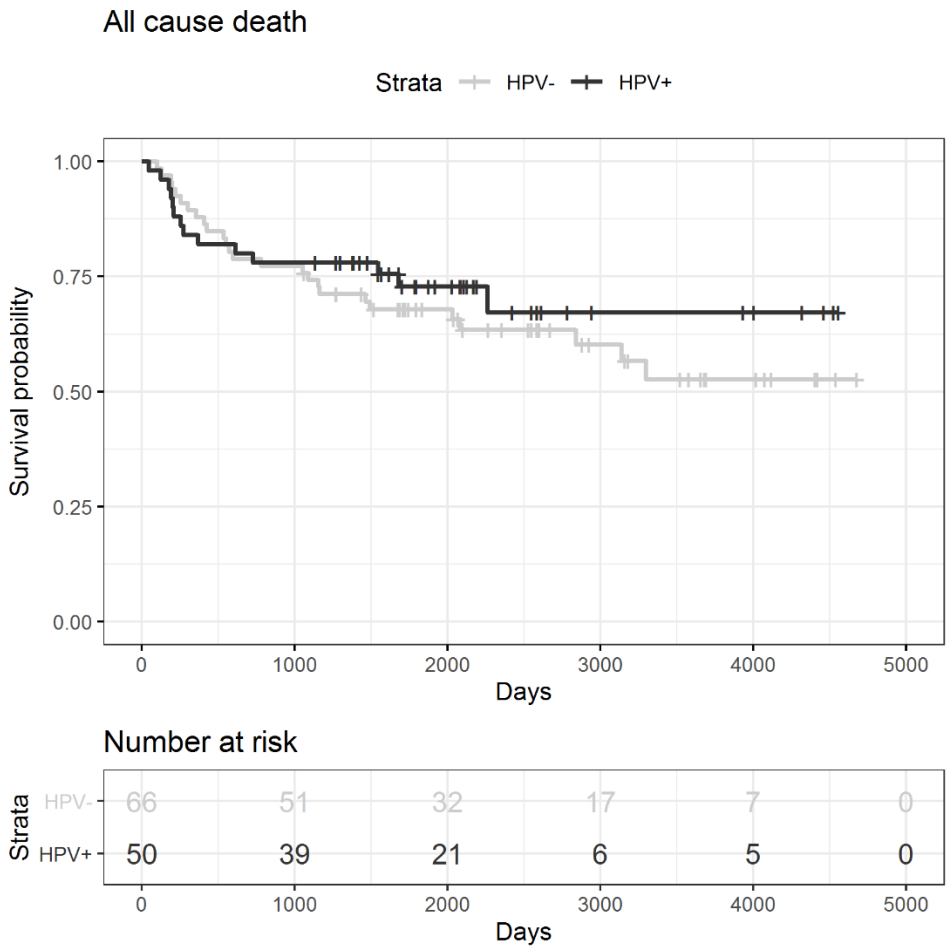
All cause death
Strata
HPV-
HPV+
Survival probability
1.00
0.75
0.50
0.25
0.00
0
1000
2000
3000
4000
5000
Days
Number at risk
Strata
HPV-
HPV+
66
51
32
17
7
0
50
39
21
6
5
0
0
1000
2000
3000
4000
5000
Days

Figure 2

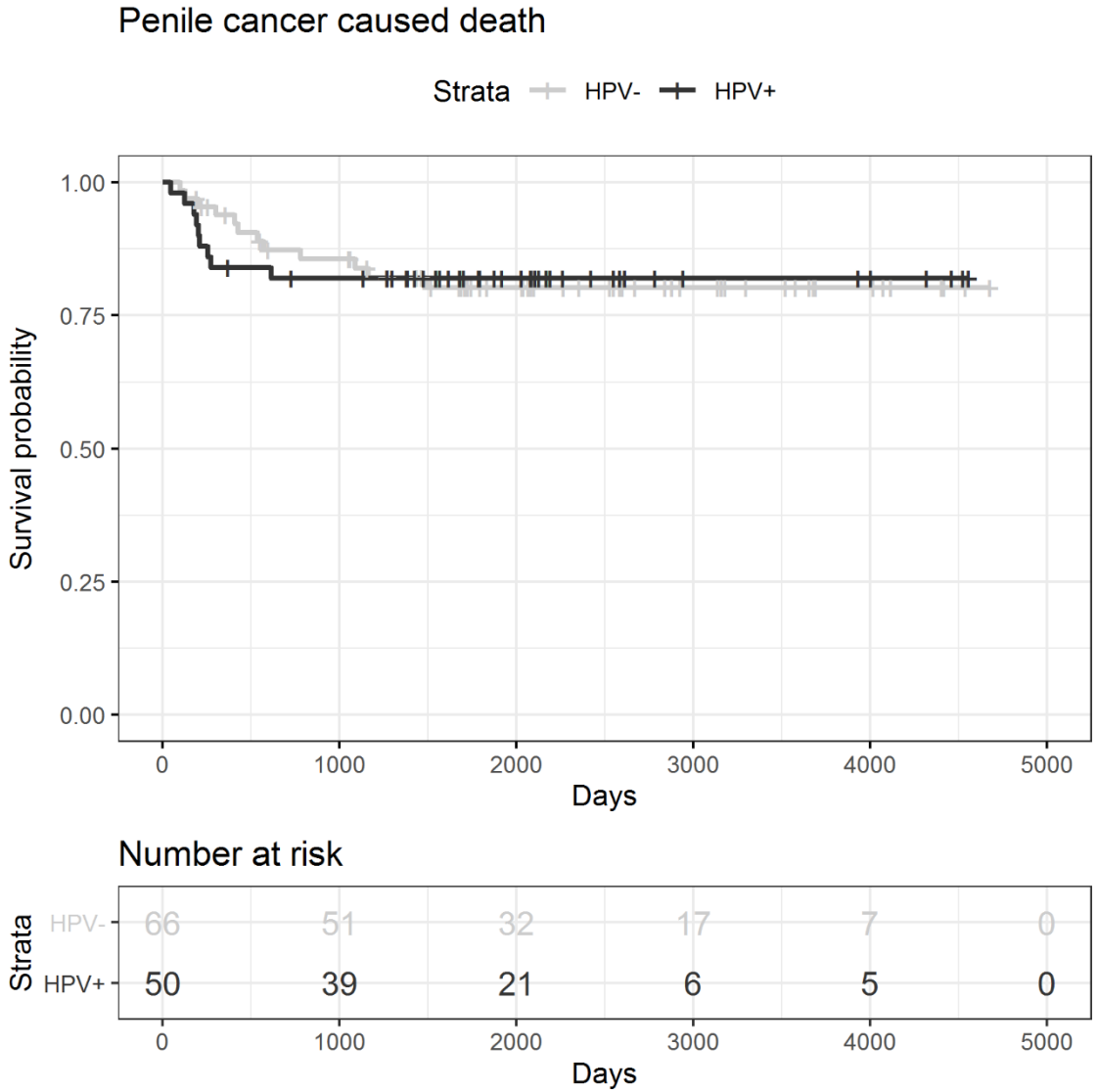
Penile cancer caused death
Strata
HPV-
HPV+
1.00
0.75
0.50
0.25
0.00
Survival probability
0
1000
2000
3000
4000
5000
Days
Number at risk
Strata
HPV-
HPV+
66
51
32
17
7
0
50
39
21
6
5
0
0
1000
2000
3000
4000
5000
Days

Figure 3