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Penile Cancer

Role of Human Papillomavirus in Penile Carcinomas Worldwide

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Abstract

Background: Invasive penile cancer is a rare disease with an approximately 22 000 cases per year. The incidence is higher in less developed countries, where penile cancer can account for up to 10% of cancers among men in some parts of Africa, South America, and Asia.
Objective: To describe the human papillomavirus (HPV) DNA prevalence, HPV type distribution, and detection of markers of viral activity (ie, E6*I mRNA and $p16^{INK4a}$) in a series of invasive penile cancers and penile high-grade squamous intraepithelial lesions (HGSILs) from 25 countries. A total of 85 penile HGSILs and 1010 penile invasive cancers diagnosed from 1983 to 2011 were included.
Design, setting, and participants: After histopathologic evaluation of formalin-fixed paraffin-embedded samples, HPV DNA detection and genotyping were performed using

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DNA
mRNA
p16
Vaccine

the SPF-10/DEIA/LiPA$_{25}$ system, v.1 (Laboratory Biomedical Products, Rijswijk, The Netherlands). HPV DNA–positive cases were additionally tested for oncogene E6*I mRNA and all cases for p16^{INK4a} expression, a surrogate marker of oncogenic HPV activity.

Outcome measurements and statistical analysis: HPV DNA prevalence and type distributions were estimated.

Results and limitations: HPV DNA was detected in 33.1% of penile cancers (95% confidence interval [CI], 30.2–36.1) and in 87.1% of HGSILs (95% CI, 78.0–93.4). The warty-basaloid histologic subtype showed the highest HPV DNA prevalence. Among cancers, statistically significant differences in prevalence were observed only by geographic region and not by period or by age at diagnosis. HPV16 was the most frequent HPV type detected in both HPV-positive cancers (68.7%) and HGSILs (79.6%). HPV6 was the second most common type in invasive cancers (3.7%). The p16^{INK4a} upregulation and mRNA detection in addition to HPV DNA positivity were observed in 69.3% of HGSILs, and at least one of these HPV activity markers was detected in 85.3% of cases. In penile cancers, these figures were 22.0% and 27.1%, respectively.

Conclusions: About a third to a fourth of penile cancers were related to HPV when considering HPV DNA detection alone or adding an HPV activity marker, respectively. The observed HPV type distribution reinforces the potential benefit of current and new HPV vaccines in the reduction of HPV-related penile neoplastic lesions.

Patient summary: About one-third to one-quarter of penile cancers were related to human papillomavirus (HPV). The observed HPV type distribution reinforces the potential benefit of current and new HPV vaccines to prevent HPV-related penile neoplastic lesions.

1. Introduction

Invasive penile cancer is a rare disease with an annual burden of 22 000 estimated cases [1]. The incidence is higher in less developed countries, where penile cancer can account for up to 10% of cancers among men in some parts of Africa, South America, and Asia [2].

Two major pathways have been described to occur in penile cancer carcinogenesis: one related to a number of penile conditions such as inflammation, phimosis, or history of lichen sclerosus and lichen planus, and another related to human papillomavirus (HPV) infection [2,3]. Circumcision acts as a protective factor, presumably by reducing HPV transmission or penile pathologic conditions associated with penile carcinogenesis [2,4].

Previous literature suggests that HPV DNA is detected in approximately half of penile cancers with variations between studies. HPV16 is the most common type detected, followed by HPV18 [5]. It remains unclear whether the differences in prevalence between studies reflect a real variation across populations or differences in sample selection or in the technology used. Given the wide range of estimates, a further insight may be gained by increasing study sample size, geographic representativeness, and standardized HPV DNA detection protocols. Additional signature of HPV activity and induced carcinogenicity such as markers of viral transcription and HPV-induced cellular transformation should be used to distinguish whether the HPV DNA detected in tumor tissue is likely an active viral infection, a transient infection, or a contaminant [6]. These additional markers are essential to get closer to the proportion of penile cancers linked etiologically to the virus.

Our objective was a comprehensive description of HPV DNA prevalence and type distribution, HPV E6*I mRNA detection, and p16^{INK4a} expression in a series of 85 penile high-grade squamous intraepithelial lesions (HGSILs) and 1010 invasive penile cancers from 25 countries.

2. Materials and methods

2.1. Study design

A retrospective cross-sectional study was designed and coordinated by the Institut Català d'Oncologia (ICO), Barcelona, Spain. Formalin-fixed paraffin-embedded (FFPE) HGSILs and invasive penile cancer specimens diagnosed from 1983 to 2011 were obtained from pathology archives in 25 countries from Europe, North America, Latin America, Africa, Asia, and Oceania (the countries are listed in Supplementary Table 1). Information about age and year of diagnosis and the original histologic diagnosis were also obtained from the participating centers.

2.2. Histopathologic evaluation

FFPE tissue blocks were processed under strict pre/post polymerase chain reaction (PCR) separation conditions to avoid potential contamination as described in a previous publication [7]. At least five FFPE sections were performed; first and last sections were used for histopathologic evaluation after hematoxylin and eosin (HE) staining. This evaluation was performed following the consensus criteria established by a panel of expert pathologists and based on schemes published by the Armed Forces Institute of Pathology and the World Health Organization [8,9]. All cases were reviewed by an expert pathologist (A.C.), and doubtful and discordant diagnoses with the original diagnosis were again reviewed by the panel to come to a specific diagnostic decision. A block was determined to be adequate for HPV DNA testing if invasive cancer or a HGSIL was observed in the two HE-stained sections of the specimen. To control for possible sources of contamination, blocks containing tissues a priori non-related to HPV infection and processed in the local pathology laboratory at the same time as the penile specimens under study were blindly processed.

2.3. HPV DNA detection and typing

DNA extraction and HPV DNA detection were previously described [7]. Briefly, HPV DNA detection was done using the SPF-10/DEIA/LiPA$_{25}$ system, v.1 (Laboratory Biomedical Products, Rijswijk, The Netherlands). The LiPA$_{25}$ detection system allows genotyping of 25 HPVs categorized by the International Agency for Research on Cancer within group 1 [10] (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), group 2A (HPV68), group 2B (HPV types 34, 53, 66, 70, and 73), and group 3 (HPV types 6 and 11), as well as other HPVs (HPV types 40, 42, 43, 44, 54, and 74). Specimens testing positive for HPV DNA by DEIA but could not be typed by LiPA$_{25}$ were further analyzed by direct Sanger sequencing of PCR products, as described in Geraets et al [11]. The cases that could not be sequenced were labeled as *HPV undetermined*. Specimens with an inconclusive probe line pattern by LiPA$_{25}$ (ie, HPV68/73 or HPV39/68/73) were also sequenced to distinguish the specific HPV types. To evaluate DNA quality, a random selection of 5% HPV DNA–negative samples were subjected to a PCR targeting the human tubulin gene and generating an amplicon of the same length as the SPF10 PCR [12]. Approximately 9% of these cases were both HPV DNA and tubulin negative, indicating poor DNA quality.

2.4. HPV E6*I mRNA detection

From 408 HPV DNA–positive cases, 368 cases underwent RNA extraction and E6*I mRNA detection. The mRNA assay targets a total of 20 HPV types (HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82) [6,13]. For each case, type-specific E6*I mRNA reverse transcriptase–PCR was performed targeting all HPV types detected at the DNA level. The detection of the cellular housekeeping gene ubiquitin C (*UBC*) was used for RNA quality control purposes in all tested cases. Testing for mRNA of all HPV DNA–negative samples was not considered due to the complexity of the assay; however, a subset of 20 HPV DNA–negative cases were tested for HPV16 E6*I mRNA splice site as a negative control (10 cases HPV DNA–negative with p16^{INK4a} downregulation and 10 cases HPV DNA–negative that showed p16^{INK4a} upregulation). All HPV DNA-negative/p16^{INK4a} negative cases were mRNA negative, whereas two of the HPV DNA-negative/p16^{INK4a} positive cases were HPV16 mRNA positive. This observation has a small impact in the overall estimates because the number of p16 upregulated cases among the HPV DNA-negative cases is small (13% [88 of 672]).

2.5. p16 ^{INK4a} expression

Immunohistochemical p16^{INK4a} expression evaluation was performed in all cases with available material, 1078 cases of 1095. The p16^{INK4a} was detected using the CINtec histology kit (clone E6H4, Roche MTM Laboratories AG, Heidelberg, Germany), following the manufacturer's protocol. A pattern of diffuse staining of >25% stained cells (nuclear and cytoplasmic) was considered positive [14].

2.6. Statistical analysis

HPV DNA prevalence was estimated among HPV DNA–analyzed cases, and HPV type–specific relative contributions were calculated among HPV DNA–positive cases. Contribution of individual types to multiple infections was calculated under a weighting attribution proportional to the prevalence for each individual type in single infections. HPV DNA prevalence and HPV type–specific detection percentages were determined globally and according to geographic regions, histopathologic categories, patient's age at diagnosis, and year of diagnosis. HPV positivity for additional markers (ie, E6*I mRNA and p16^{INK4a}) was also estimated. The chi-square test and Student *t* test were used to evaluate associations between variables and HPV DNA, E6*I mRNA, and p16^{INK4a} positivity.

Agreement between HPV DNA detection and p16^{INK4a}, and mRNA and p16^{INK4a} expression was assessed by the κ score. Prevalence-adjusted Bias-adjusted Kappa (PABAK) was also estimated. The McNemar test for matched-pair data was used for assessing unequal distribution of discordant results.

Statistical significance for all analyses was set at the two-sided 0.05 level. Data analyses were performed with SPSS software v.13.0 (IBM Corp., Armonk, NY, USA) and with Stata software v.10.0 (Stata Corp., College Station, TX, USA).

2.7. Ethical considerations

Specimens were received anonymously. All protocols were approved by international and ICO ethics committees.

3. Results

A total of 85 HGSILs and 1010 invasive penile cancers were HPV DNA analyzed and included in the final analyses. Figure 1 shows the algorithm of the study.

Patients with a HGSIL diagnosis were approximately 10 yr younger than patients diagnosed with invasive penile carcinoma (mean age at diagnosis: 54.8 yr, standard deviation [SD]: 18.4 for HGSILs vs 64.1 yr [SD: 15.0] for invasive cancer cases; $p < 0.001$). There was a higher representation from European and Latin American countries (almost 90% of the cases) and from the period of diagnosis 2000 to 2011, in both preinvasive and invasive lesions (Table 1). Penile HGSILs displayed warty-basaloid morphologic features more frequently (78.8%); while, in invasive penile cancer, the most frequent histologic type was squamous cell carcinoma (SCC) without warty-basaloid features (63.5%) (Table 2). Less frequently we identified warty-basaloid SCC (22.9%), mixed warty-basaloid and non–warty-basaloid histologic SCC cases (7.3%) and "other" diagnoses (6.3%).

HPV DNA prevalence was 87.1% (95% confidence interval [CI], 78.0–93.4) in HGSILs and 33.1% (95% CI, 30.2–36.1) in invasive penile cancers (Table 1). No statistically significant differences were observed for age at diagnosis or for period of diagnosis, even when data were analyzed using shorter calendar periods or smaller age intervals. Within penile invasive cancers, HPV prevalence varied by geographic region with the highest prevalences in Africa, Latin America, and Europe, and the lowest in Asia. HPV DNA prevalence showed variations according to the histologic diagnosis both in penile HGSILs and invasive cancers with the highest prevalence in lesions with warty-basaloid features and the lowest in SCCs without warty-basaloid features (Table 2).

Among HPV DNA-positive samples (Table 3), the percentage of multiple infections was higher for penile HGSILs (17.6%) than for invasive cancers (9.0%) ($p = 0.027$). The most frequent HPV type was HPV16 for both penile HGSILs (79.6% including multiple infections) and invasive cancers (68.7%). Among invasive cancers, the second most common type was HPV6 (3.7%). HPV16 and 18 accounted together for approximately 70% of penile invasive cancers. The detailed HPV type distribution by the different variables considered (geographic region, period, age at diagnosis, and histologic diagnosis) is described in detail in Supplementary Tables 2–9.

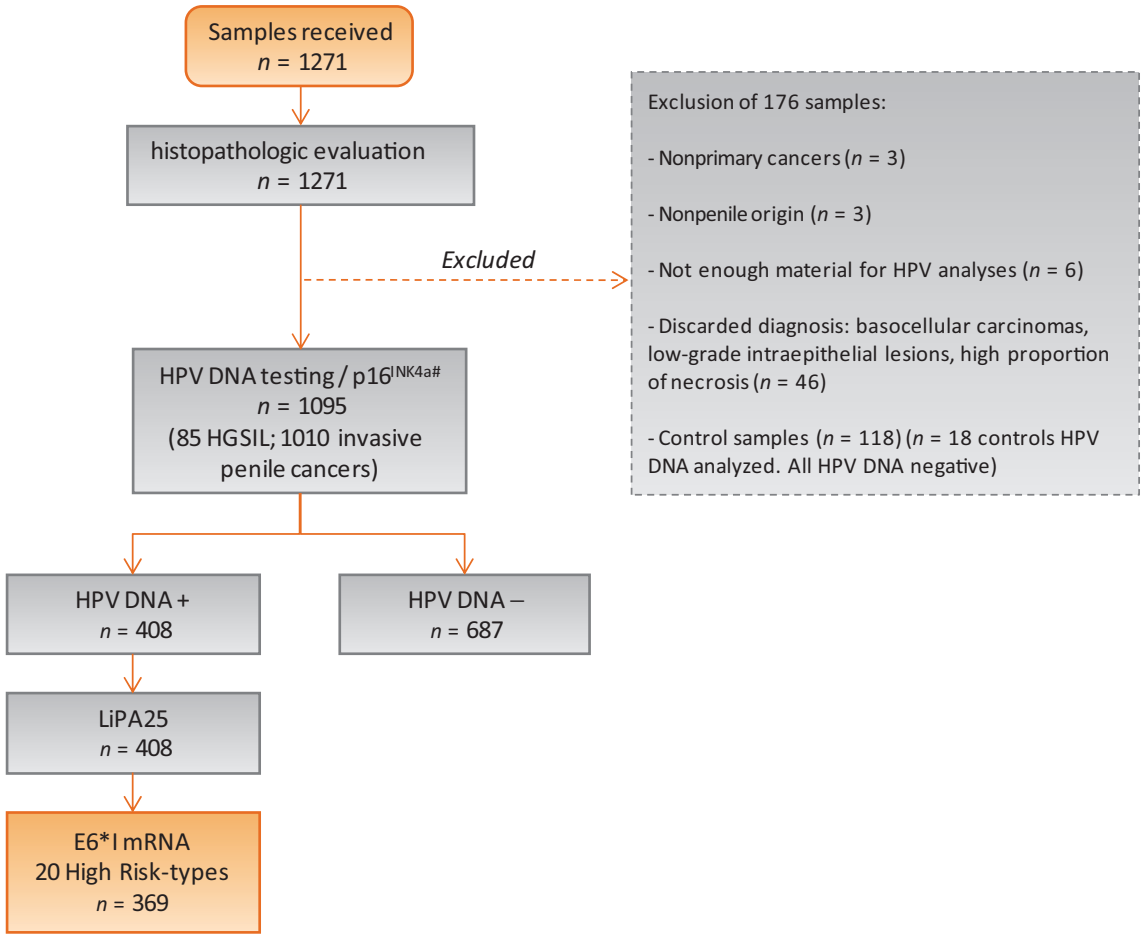

Fig. 1 – Study algorithm. #p16^{INK4a} was performed in 1078 cases; in 17 cases there was not available material to perform the staining. HGSIL = high-grade squamous intraepithelial lesion; HPV = human papillomavirus.

Table 1 – Sample description and human papillomavirus DNA prevalence in penile high-grade squamous intraepithelial lesions and invasive penile cancers

	Penile HGSIL					Invasive penile cancer				
			HPV DNA prevalence					HPV DNA prevalence		
	n	%	*n*	%	95% CI	*n*	%	*n*	%	95% CI
Region										
Europe	64	75.3	57	89.1	78.8–95.5	419	41.5	135	32.2	27.8–36.9
North America	–	–	–	–	–	16	1.6	3	18.8	4.0–45.6
Latin America	11	12.9	7	63.6	30.8–89.1	480	47.5	175	36.5	32.1–40.9
Africa	–	–	–	–	–	19	1.9	7	36.8	16.3–61.6
Asia	4	4.7	4	100.0	39.8–100.0*	67	6.6	9	13.4	6.3–24.0
Oceania	6	7.1	6	100.0	54.1–100.0*	9	0.9	5	55.6	21.2–86.3
Period of diagnosis										
1983–1999	20	23.5	17	85.0	62.1–96.8	298	29.5	88	29.5	24.4–35.1
2000–2011	65	76.5	57	87.7	77.2–94.5	711	70.4	245	34.4	31.0–38.1
Missing	–	–	–	–	–	1	0.1	1	100.0	2.5–100.0*
Age at diagnosis, yr										
<55	41	48.2	38	92.7	80.1–98.5	246	24.4	88	35.8	29.8–42.1
55–75	30	35.3	24	80.0	61.4–92.3	435	43.1	140	32.2	27.8–36.8
>75	13	15.3	11	84.6	54.6–98.1	222	22.0	75	33.8	27.6–40.4
Missing	1	1.2	1	100.0	2.5–100.0*	107	10.6	31	29.0	20.6–38.5
TOTAL	85	100.0	74	87.1	[78.0–93.4]	1,010	100.0	334	33.1	30.2–36.1

CI = confidence interval; HGSIL = high-grade squamous intraepithelial lesion; HPV = human papillomavirus; HPV DNA prevalence indicates HPV DNA positivity.
* One-sided 97.5% CI. Statistically significant association was observed for HPV DNA positivity and geographic region in invasive penile cancer.

Table 2 – Histologic diagnosis and human papillomavirus DNA prevalence in penile high-grade squamous intraepithelial lesions and invasive penile cancers

			HPV DNA prevalence		
	n	%	*n*	%	95% CI
HGSIL					
Warty-basaloid	67	78.8	65	97.0	89.6–99.6
Differentiated	13	15.3	4	30.8	9.1–61.4
Mixed	5	5.9	5	100.0	47.8–100.0*
Invasive penile cancer					
SCC 100% warty basaloid	231	22.9	174	75.3	69.4–80.7
SCC 100% non–warty-basaloid	641	63.5	94	14.7	12.0–17.6
SCC mixed histologies	74	7.3	33	44.6	33.0–56.6
Other†	64	6.3	33	51.6	38.7–64.2

CI = confidence interval; HGSIL = high-grade squamous intraepithelial lesion; HPV = human papillomavirus; HPV DNA prevalence = HPV DNA positivity; SCC = squamous cell carcinoma.
* One-sided 97.5% CI.
† Other histologic diagnoses includes 30 undifferentiated carcinomas, 19 pseudoepithelial, 7 pseudoadenomatous, 7 condylomas with invasive component, 1 adenosquamous. Statistically significant association was observed for HPV DNA positivity and histologic diagnosis either in HGSIL and invasive penile cancer.

Table 3 – Human papillomavirus (HPV) type–specific relative contribution among HPV DNA–positive penile high-grade squamous intraepithelial lesions and invasive penile cancers

	Penile HGSIL (HPV+, *n* = 74)				Invasive penile cancer (HPV+, *n* = 334)				Relative contribution	
	Single*		Single and multiple†‡		Single*		Single and multiple†		Ratio (cancer to HGSIL)**	
HPV type	*n*	%	*n*	%	*n*	%	*N*	%	Ratio	95% CI
HPV6	–	–	–	–	12	3.6	12	3.7	–	–
HPV11	1	1.4	1	1.4	4	1.2	5	1.5	1.11	0.13–9.34
HPV16	51	68.9	59	79.6	210	62.9	229	68.7	**0.86**	**0.75–0.99**
HPV18	–	–	–	–	4	1.2	5	1.5	–	–
HPV26	–	–	–	–	2	0.6	2	0.6	–	–
HPV27	–	–	–	–	1	0.3	1	0.3	–	–
HPV30	–	–	–	–	2	0.6	2	0.6	–	–
HPV31	1	1.4	1	1.8	2	0.6	3	0.8	0.66	0.07–6.30
HPV32	–	–	–	–	2	0.6	2	0.6	–	–
HPV33	3	4.1	4	5.5	8	2.4	10	2.9	0.55	0.18–1.72
HPV35	–	–	–	–	9	2.7	9	2.7	–	–
HPV39	–	–	–	–	2	0.6	2	0.7	–	–
HPV40	–	–	–	–	1	0.3	1	0.3	–	–
HPV42	–	–	–	–	1	0.3	1	0.3	–	–
HPV43	–	–	–	–	1	0.3	1	0.3	–	–
HPV45	–	–	–	–	9	2.7	9	2.7	–	–
HPV51	–	–	–	–	2	0.6	3	0.8	–	–
HPV52	–	–	–	–	4	1.2	5	1.5	–	–
HPV53	–	–	–	–	2	0.6	2	0.6	–	–
HPV55	–	–	–	–	1	0.3	1	0.3	–	–
HPV56	–	–	–	–	2	0.6	2	0.6	–	–
HPV58	2	2.7	3	3.7	3	0.9	4	1.3	0.30	0.07–1.29
HPV59	–	–	–	–	4	1.2	5	1.6	–	–
HPV61	1	1.4	1	1.4	–	–	–	–	–	–
HPV66	–	–	–	–	1	0.3	1	0.3	–	–
HPV68	–	–	–	–	1	0.3	1	0.3	–	–
HPV70	–	–	–	–	1	0.3	1	0.4	–	–
HPV73	–	–	–	–	3	0.9	3	0.9	–	–
HPV74	–	–	–	–	2	0.6	3	1.0	–	–
HPV76	–	–	–	–	1	0.3	1	0.3	–	–
HPV82	–	–	–	–	1	0.3	1	0.3	–	–
HPV undetermined	2	2.7	2	2.7	6	1.8	6	1.8	0.66	0.14–3.23
Multiple	13	17.6			30	9.0			**0.51**	**0.28–0.93**

CI = confidence interval; HGSIL = High-grade squamous intraepithelial lesion; HPV = human papillomavirus; HPV+ = HPV DNA positive.
95% CI is highlighted in bold numbers when it does not contain 1.
* Single and multiple infections counted separately.
† Multiple infections were added to single types under a weighting attribution proportional to the detection found in cases with single types, as described in the methodology.
‡ Three multiple infections were not counted in the proportional attribution estimation because the HPV types were not found as single infections: HPV6/73, HPV43/52, and HPV51/52.
** Considering "single and multiple" columns estimation.

Table 4 – Human papillomavirus (HPV) DNA, E6*I mRNA, and p16^{INK4a} by HPV types (single infections), among HPV DNA–positive penile high-grade squamous intraepithelial lesions and invasive penile cancers

	DNA+		mRNA+		p16+		mRNA+/ p16+		mRNA+/ p16−		mRNA−/ p16+		mRNA−/ p16−	
HPV type	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Penile cancer														
HPV16*	210	62.9	179/207	86.5	172/205	83.9	158/202	78.2	16/202	7.9	11/202	5.4	17/202	8.4
Any high-risk*	272	81.4	227/266	85.3	212/265	80.0	191/259	73.7	29/259	11.2	16/259	6.2	23/259	8.9
High-risk (excluding HPV16)*	62	18.6	48/59	81.4	40/60	66.7	33/57	57.9	13/57	22.8	5/57	8.8	6/57	10.5
No high-risk*	26	7.8	NA	NA	4/26	15.4	NA	NA	NA	NA	NA	NA	NA	NA
HPV undetermined	6	1.8	NA	NA	3/6	50.0	NA	NA	NA	NA	NA	NA	NA	NA
Multiples	30	9.0	24/29	82.8	22/30	73.3	21/29	72.4	3/29	10.3	1/29	3.4	4/29	13.8
TOTAL	334	-	251/295	85.1	241/327	73.7	212/288	73.6	32/288	11.1	17/288	5.9	27/288	9.4
HGSIL														
HPV16*	51	68.9	49/51	96.1	42/47	89.4	41/47	87.2	5/47	10.6	1/47	2.1	0/47	0.0
Any high-risk*	57	77.0	55/57	96.5	45/51	88.2	44/51	86.3	6/51	11.8	1/51	2.0	0/51	0.0
High-risk (excluding HPV16)*	6	8.1	6/6	100.0	3/4	75.0	3/4	75.0	1/4	25.0	0/4	0.0	0/4	0.0
No high-risk*	2	2.7	NA	NA	0/2	0.0	NA	NA	NA	NA	NA	NA	NA	NA
HPV undetermined	2	2.7	NA	NA	0/2	0.0	NA	NA	NA	NA	NA	NA	NA	NA
Multiples	13	17.6	13/13	100.0	8/13	61.5	8/13	61.5	5/13	38.5	0/13	0.0	0/13	0.0
TOTAL	74	-	68/70	97.1	53/68	77.9	52/64	81.3	11/64	17.2	1/64	1.6	0/64	0.0

HGSIL = high-grade squamous intraepithelial lesion; HPV = human papillomavirus; NA = not applicable, not tested for mRNA.
High-risk types: Risk groups were defined according to the last International Agency for Research on Cancer classification; HPV types included in group 1, group 2A, and group 2B were considered high-risk; other HPV types were classified as no high-risk HPV types [10]. mRNA and p16 concordance: penile cancer: total concordance: 83%; prevalence-adjusted bias-adjusted kappa (PABAK) estimate: 0.660; McNemar test: 0.044; penile high-grade squamous intraepithelial neoplasia: total concordance: 81%; PABAK estimate: 0.646; McNemar test: 0.006.
* Only single infections are counted.

p16^{INK4a} expression and presence of HPV DNA, and mRNA showed good overall concordance and agreement (Supplementary Tables 10 and 11). Table 4 presents a summary of HPV DNA, E6*I mRNA, and p16^{INK4a} positivity by HPV16, high-risk types, other HPV types, multiple and undetermined infections in penile HGSILs, and invasive cancers. In summary, HPV E6*I mRNA detection in high-risk types was high in both types of lesions: in HGSILs (97.1%) and in invasive penile cancer (85.1%). Globally, the proportion of p16^{INK4a} upregulation was also high and showed a trend by HPV grouped types: higher for HPV16 than high-risk HPV types excluding HPV16, being the lowest in non–high-risk HPVs ($p < 0.05$). Similar to results for HPV DNA, the only associated factors with mRNA and/or p16 detection were geographic region and histologic diagnosis (data not shown). Supplementary Tables 12 and 13 show the detailed results of HPV E6*I mRNA and p16^{INK4a} expression for each of the cases harboring multiple infections at the HPV DNA level. Globally, in all series, the prevalence of p16^{INK4a} upregulation and HPV E6*I mRNA detection in addition to HPV DNA positivity was observed in 69.3% of HGSILs and at least one of these HPV activity markers in 85.3%. In penile cancers, these figures were 22.0% and 27.1%, respectively.

4. Discussion

To our knowledge this is the largest study to evaluate the role of HPV infection in penile cancer comprehensively by using three different markers of viral presence and activity at the level of viral DNA, mRNA, and cell protein expression, p16. HPV DNA was detected in 33.1% of 1010 penile cancers and in 87.1% of 85 HGSILs. One additional marker of viral activity, p16^{INK4a} upregulation or oncogene mRNA detection, was observed in most of the HPV DNA–positive cases. HPV16 was the most frequent HPV type detected in both cancers and HGSILs followed by HPV6.

Our results show that prevalence of the three markers in penile cancers depends on both the geographic origin as well as on the specific histologic presentation of the cancer. The HPV DNA prevalence reported here for penile invasive cancers (33.1%) is lower than that found in systematic reviews by Miralles-Guri and coworkers (47%) [5], Backes and coworkers (50%) [15], or a 2014 study of penile cancers from US (63%) [16]. The observed differences could be due to the wide variation of small studies with different detection methods included in the systematic reviews, compared with our study with a large number of samples and well-standardized virus detection protocols. Differential selection in the geographic origin of cases and contribution of histologies (ie, reporting bias) could also explain the differences. Regarding geography, in our study the lowest HPV DNA detection was found in Asia (13.4%) in sharp contrast to the 55.3% reported by Miralles-Guri and coworkers for this geographic region [5]. It is important to note that countries contributing to both reports did not overlap. Differences in HPV exposure and other factors such as differential circumcision prevalence across countries

Table 5 – Possible interpretation of markers combination in high-risk human papillomavirus types

HPV DNA	HPV mRNA	p16	HPV driven	Interpretation
+	+	+	Yes	Full pattern of HPV oncogens transcription and HPV-transformation by interaction of HPV oncoproteins with cellular proteins (eg, p16)
+	+	–	Yes	Partial pattern: p16 negativity explained, for example, by p16 gen methylation, loss of p16 in tumor cells as a result of increasing genetic and epigenetic chromosomal instability, etc
+	–	+	Yes	Partial pattern: mRNA negativity explained by possibly false-negative mRNA detection due to low HPV mRNA levels, below the detection level of the E6*I mRNA assays, etc
+	–	–	No	Nononcogenic HPV infection; only HPV/DNA detection with no additional viral activity marker

HPV = human papillomavirus.

could explain these geographic differences. Regarding histologic diagnosis, cases with warty-basaloid morphologic features are strongly related to HPV compared with other histologies. We note that in the present study, cases were requested to be nonselected series preferably randomly selected or consecutive in time, to ensure the representativeness at each of the participating centers. It is important to be cautious and not to consider overall prevalence as universal because the role of HPV in penile cancer etiology could be strongly influenced by histologic distribution and geographic region as is also true for other HPV malignancies such as vulvar and head and neck cancers [1,17].

Although an increase of the HPV DNA prevalence in recent years was observed for penile cancer, we could not identify a significant association, contrary to other reports [16]. Also, we could not identify an age effect by which HPV-related penile tumors were diagnosed in younger patients than non–HPV-related penile tumors as has been reported for head and neck and vulvar cancers. One of the possible explanations for this observation is that immune response to HPV infection works differently in the external male genitalia than in female genitalia or head and neck sites. It has been described that penile immune response to HPV infection is lower when compared with other anatomic sites and that baseline HPV seropositivity in men does not positively correlate with a reduced risk of subsequent HPV16 acquisition [18,19].

The most frequent HPV type in both penile HGSILs and invasive cancer was HPV16 (79.6% and 68.7%, respectively), in good agreement with previous reports [5]. HPV16 and 18 accounted together for an approximately 70% of HPV DNA–positive penile cancers. The nine HPV types included in the new 9-valent Gardasil vaccine (HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58; Merck & Co, Inc, Kenilworth, NJ, USA), recently approved by the US Food and Drug Administration, showed a combined prevalence among HPV DNA–positive cases of 92% in penile HGSILs and 85% in invasive cancers. It is important to highlight that the detection rates of low-risk HPV types (eg, HPV6 and HPV11) were higher in penile cancers (3.7% and 1.5%, respectively) compared with those observed in other HPV-related anogenital cancers. Indeed, HPV6 was the second most common type among penile cancers (3.7%). To further investigate the potential causal association, a subset of eight penile cancer cases with single HPV6 infection and three with single HPV11 infection were further analyzed by laser capture microdissection [20]. In 75% of cases (six of eight), HPV6 DNA was found in tumor cells and 33% (one of three) in HPV11 cases, thus confirming the initial detection in the whole tissue section and excluding potential contamination from adjacent tissue. These results thus confirm that low-risk types can be associated with invasive penile neoplastic lesions.

Overall, 17% of penile HGSILs harbored HPV DNA from multiple HPV types compared with 9% in penile cancers. This decrease in multiplicity of infection with neoplastic disease progression has been also described for other anogenital cancers, and it may reflect the concept of clonal development of invasive neoplasia resulting from persistent infection with a single HPV type [21]. Regarding the monoclonal concept, it is interesting to highlight that in all cases harboring multiple infections at the DNA level in which more than one mRNA type was detected, there was always more than one histologic neoplastic lesion (ie, low-grade squamous intraepithelial lesion [LGSIL] with HGSIL, and LGSIL or HGSIL adjacent to the invasive cancer), suggesting that each type could be linked to a different histologic lesion.

In the large majority of penile HGSILs and invasive cancers, an additional marker of HPV viral activity (E6*I mRNA, $p16^{INK4a}$) to the HPV DNA positivity was identified, suggesting that the virus was active and involved in the oncogenic process and not a mere passerby infection (Table 5). Regarding $p16^{INK4a}$ expression, it is worth noting that cases harboring a low-risk type tend to show a lower $p16^{INK4a}$ upregulation than high-risk types. A similar finding was reported by Guimerà and colleagues and may be related to the lack of degradation of pRB by the E7 viral protein from low-risk HPVs that does not trigger p16 upregulation [20].

The value of the present study compared with previous reports is the large series of cases evaluated using a highly sensitive HPV DNA detection and genotyping system (SPF-10/DEIA/$LiPA_{25}$) under a thorough contamination control process in a single central laboratory, together with the addition of other markers to evaluate the causal association of HPV in penile lesions (ie, detection of HPV E6*I mRNA and the evaluation of a surrogate marker of HPV-associated cellular transformation [$p16^{INK4a}$]). The study, however, has some limitations such as the lack of individual information regarding other risk factors and clinical or follow-up data.

5. Conclusions

One-third to one-quarter of penile cancers were related to HPV when considering HPV DNA detection alone or adding

at least one HPV activity marker, respectively. HPV16 was the most frequent HPV type detected, accounting for 70% of HPV DNA–positive penile cancers when combined with HPV18. This figure rose to 85% when considering additional types included in the 9-valent Gardasil vaccine. The observed HPV type distribution reinforces the potential benefit of current and new HPV vaccines in the reduction of HPV-related penile neoplastic lesions.

Author contributions: Laia Alemany had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2015.12.007.

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