European Review for Medical and Pharmacological Sciences 2015; 19: 4153-4163

Analysis of persistence of human papillomavirus infection in men evaluated by sampling multiple genital sites

G. CAPRA[1], A.G. NYITRAY[2], B. LU[3], A. PERINO[1], R. MARCI[4], R. SCHILLACI[1], D. MATRANGA[1], A. FIRENZE[1], M. CALECA[1], C. BELLAVIA[1], F. GUARNERI[1], A. GIULIANO[3], L. GIOVANNELLI[1]

[1]Department of Sciences for Health Promotion and Mother-Child Care "G. D'Alessandro", "Paolo Giaccone" Polyclinic University Hospital, Palermo, Italy
[2]Center for Infectious Disesases, University of Texas School of Public Health at Houston Tx, Houston, Texas, USA
[3]Department of Cancer Epidemiology, Center for Infection Research in Cancer, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA
[4]Department of Morphology, Surgery and Experimental Medicine, Section of Obstetrics and Gynecology, University of Ferrara, Ferrara, Italy

Abstract. – OBJECTIVE: Although human papillomavirus (HPV) infection has been studied extensively in women, data on male infection are limited. The purpose of this study was to investigate persistence of HPV infection at multiple genital sites in men and to define potential associations with socio-behavioural characteristics.

PATIENTS AND METHODS: Penile, urethral and seminal specimens were tested by the INNO-LiPA HPV system (Innogenetics) and a PCR assay. Persistence was defined as the detection of the same HPV type at ≥ 2 consecutive visits. The Kaplan-Meier method and the log-rank test were applied to estimate the likelihood of persistence.

RESULTS: A total of 50 men (median age: 33 years) were followed for a median of 14.7 months. Altogether, 49%, 36%, 26% and 11% of baseline HPV-positive men had 6-, 12-, 18- and 24-month persistent infection with any HPV type, respectively. The 6-, 12- and 18- month persistence was more common for oncogenic HPV infections; 24-month persistence was similar. The median duration of persistence was 21.7 months for any HPV. The median duration of persistence for any HPV type was significantly longer in the penile sample (22.5 months, 95% CI: 18.3-26.7) than the semen sample (15.3 months, 95% CI: 14.5-16.1).

CONCLUSIONS: Over a third of type-specific HPV infections in men remained persistent over a 24-month period. The median duration of HPV infection was longer in penile samples compared to seminal samples. With increasing attention on HPV vaccination as a potential preventive approach for men, it is imperative to obtain additional insight on natural history of HPV infection in men, particularly as far as incidence and duration are concerned.

Key Words:
HPV infection, Genital sampling, Men, Persistence, Multiple sampling.

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted viral infection and is associated with ano-genital and oral disease in women and men[1,2]. There are approximately 45 HPV types that infect the genital mucosa and are classified as oncogenic or non-oncogenic HPV types on the basis of their association with pre-malignant and malignant genital lesions[3]. In women, most HPV infections are asymptomatic and clear spontaneously within 1-2 years[4]; however, some oncogenic infections persist and are etiologically linked with cervical cancer[5,6].

In contrast with the wealth of data available in women, much less is known about HPV infection in men. However, since the risk of HPV infection and cervical cancer in females is significantly influenced by male sexual behaviour[7], a greater understanding of the natural history of HPV infection in men is critical in cervical cancer prevention. This knowledge is also needed to inform policy decisions concerning the prevention and treatment of HPV-related disease in men, e.g. cost-effectiveness models for HPV vaccination.

The persistence of genital HPV infection in men has been evaluated in few prospective studies[6-11]. Most of these studies have examined pe-

Corresponding Author: Lucia Giovannelli, MD; e-mail: lucia.giovannelli@unipa.it

nile HPV infection. However, since HPV infection in men is often multifocal, information on the characteristics of infections at multiple genital sites can provide insight on the dynamics of genital HPV infection in men[8,14,15]. In addition, given the wide heterogeneity of HPV types infecting the male genital tract[16,17], and the variable sensitivity of different molecular methods available for HPV detection and typing[18,19], the combined use of complementary testing methods might considerably improve the sensitivity of HPV detection.

The current study investigated the persistence of type-specific HPV infection at multiple genital sites over a 24-month period using a combination of two highly-sensitive HPV testing methods and then examined the potential association with selected socio-behavioural characteristics.

Patients and Methods

Study Population

From January 2006 through April 2007, participants were recruited from men who requested an HPV test at the Unit of Virology of the Department of Sciences for Health Promotion and Mother and Child Care (Polyclinic, Palermo University, Palermo, Italy). The common reasons for requesting an HPV test included having an HPV-positive partner or having a risky sexual contact in the prior 2 months. A total of 65 men aged 18 years or older who tested HPV DNA positive were subsequently invited to participate in a longitudinal study of HPV infection in men. Men were enrolled in the study if they planned to stay in the area for the next 2 years, consented to a medical and sexual history interview and a genital sampling at each study visit for a maximum of 2 years, and agreed to be re-sampled in cases of any initially inadequate genital sampling, as indicated by the absence of β-globin in the genital DNA sample. Participants were followed at 6-month intervals until clearance of existing HPV infection or for a maximum of 24 months whichever came first. All participants gave written consent prior to entering the study. The informed consent and the study protocol were approved by the institutional Review Board at the Polyclinic, Palermo University (Italy).

Data Collection and Genital Sampling

At each study visit, the study clinicians administered a medical and sexual-history interview to collect information on sociodemographic characteristics, reproductive history and sexual behaviour. In addition, a genital examination was performed to identify signs of flat, macular, papular, and acuminate lesions. For HPV sampling, patients were instructed to avoid washing their genitalia the day before the examination and to observe 2 days of sexual abstinence before the examination. Three samples were collected from penis, urethra and semen. Genital sampling was performed as described previously[14], with some modifications. In brief, for penile sampling, cells from the dorsal/ventral surface of the shaft were collected by a standard-sized, dry Dacron swab first, and then by a saline-wetted Dacron swab. Cells from the inner foreskin, coronal sulcus, frenulum and glans were also collected using a saline-wetted cytobrush. Five to six back-and-forth movements of the swab/cytobrush were performed at each penile site. All penile cells were subsequently combined and placed in a vial with 3 ml of phosphate-buffered saline. For urethral sampling, a very thin, saline-wetted cytobrush was inserted 1.5 cm into the urethra, rotated 360 degrees, and removed. The urethral cells were placed in a separate vial with 3 ml of phosphate-buffered saline. The semen sample was obtained through participant self-masturbation and placed in sterile containers. If the sample was obtained at home, men were asked to transfer the sample to the laboratory within 2 hours after ejaculation. All three samples were processed for DNA extraction immediately following collection.

DNA Extraction and HPV Testing

Cells obtained from penile brushing and urethral brushing were spun down at 13,000 rpm for 5 min and total DNA was extracted with the use of the High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany), following manufacturer's instructions. The semen samples were maintained at room temperature until complete liquefaction (if the sample was very viscous, it was maintained at 37°C for 10 to 15 min). Two aliquots of 200 μl of semen were then extracted by using the same kit as for the penile and urethral samples (Roche), except that the incubation for proteinase K digestion was at 56°C for 60 min and the final elution in 100 μl.

DNA quality and the absence of inhibitors were confirmed by testing for the human β-globin gene, as described elsewhere[14]. If a participant was negative for β-globin, he was resam-

pled within one week, resulting in all men being β-globin positive.

Amplifications were carried out in a DNA thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany) and the PCR products were analyzed in 8% polyacrylamide gel. The presence of HPV DNA was detected using two HPV assays. Initially, the INNO-LiPA HPV Genotyping kit (Innogenetics N.V., Ghent, Belgium), based on the combined use of SPF_{10} PCR and LiPA hybridication[20], was employed. The SPF general primers detect at least 43 different HPV genotypes[20] and the LiPA type-specific assay identifies twenty-eight types. Due to the higher number of HPV types detected by the SPF_{10} primers than the LiPA assay, some samples yielded SPF_{10}-positive/LiPA-negative results. These HPV types were subsequently amplified by a highly sensitive *nested* PCR assay, consisting of a first step of amplification with the MY09/11 primer pair, followed by the second step with the GP05+/GP06+ primers, as previously described[18]. The HPV genotyping procedure was based on the direct sequencing of MY/GP-PCR fragments, utilizing consensus nested primers as sequencing primers, as described elsewhere[18]. Briefly, the amplification products were purified by Microcon YM-100 Filter Devices (Amicon; Millipore, Billerica, MA, USA), and approximately 5 ng of product was added to 4 μl of BigDye™ Terminator Ready Reaction mix (Applied Biosystems, Foster City, CA, USA). The purification of reaction mixtures and removal of free BigDye™ was performed by Centrisep Spin Columns (Princeton Separations, Adelphia, NJ, USA) and the mixture was analyzed on an ABI PRISM1 310 Genetic Analyzer (Applied Biosystems). Alignments were obtained from the online BLAST server.

Statistical Analysis

HPV types were classified by their oncogenic potential[3,6] with 17 types classified as oncogenic HPV (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70 and 73), and the rest classified as non-oncogenic types. Oncogenic infections were defined as infections with oncogenic HPV types, regardless of co-infection with non-oncogenic types, and further divided to infections with a single or multiple oncogenic types. Non-oncogenic HPV infections are comprised of infection with a single or multiple non-oncogenic genotypes only.

Persistence of type-specific HPV infection was defined as the detection of the same HPV genotype in the same subject in 2 or more consecutive visits, regardless of the anatomical origin of the samples. Persistent infections that were either first detected at the baseline or acquired during the follow-up were included. It was classified as 6-, 12-, 18- or 24- month persistence according to the minimal length of HPV presence in the same subject. Overlapping persistence categories were used, and men with longer-term persistence were also counted in shorter-term persistence (e.g., a man who had a 12-month persistent infection was also counted as a case for 6-month persistence). Given that persistence was defined on a type-specific basis, a man could contribute multiple persistence events. An intermittent negative HPV result between two positive results (e.g., positive-negative-positive) was treated as false-negative and, thus, re-assigned as positive.

The proportion of men with persistence of different durations was calculated using the number of men who were positive for an HPV type of interest at the baseline or during follow-up (excluding infection detected at the last documented visit) as the denominator. The proportion thus represented the percent of men who developed persistent infection among those who were ever infected with the corresponding HPV type during the study period. The Kaplan-Meier method was applied to estimate median durations of persistence and its 95% confidence intervals for any, oncogenic and non-oncogenic HPV infections. The difference in the median duration of persistence between oncogenic and non-oncogenic HPV infections was considered statistically significant in the presence of non-overlapping 95% confidence intervals. Estimates were obtained for persistent infections detected in any sample (penile, urethral or semen sample) and in each type of sample, respectively. The associations between HPV persistence and baseline risk factors were assessed through univariate analysis in which the Kaplan-Meier survival probabilities stratified by individual risk factors were compared using the log-rank test (p-value < 0.05 statistically significant). All statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and Stata/MP v. 11.2 (StataCorp LP, College Station, TX, USA).

Results

Of 65 men positive for genital HPV at the baseline, 50 were enrolled in the study, corre-

sponding to a 77% participation rate. On average each participant contributed 3.4 visits, amounting to 170 total visits. The median follow-up time was 14.7 months (range: 4.8-26.9). The subsequent loss to follow-up was 6%. Characteristics of the study participants are shown in Table I.

The mean age of participants at enrolment was 34.6 years (median: 33.0; range: 20-60). The majority of men reported > 5 lifetime sex partners (72%), having a current partner for 12 months or longer (56%), and were current smokers (58%). Seven men (14%) had exophytic genital warts. The majority of men were sexual partners of women with confirmed cervical HPV infection (86%). There were no differences between the study participants and the 15 men who declined participation – by age, HPV status in sexual partner, having a current sexual partner, or presence of external genital warts in men or in sexual partner; however, the groups did differ somewhat by reported history of sexually transmitted disease, with non-participants being more likely to report a sexually transmitted disease (53% vs. 24%; p = 0.05).

The rate of persistence of different durations for group and type-specific HPV infections is shown in Table II. With all genital samples combined, persistent infection of ≥ 6, 12, 18 and 24 months with any HPV type was detected in 49%, 36%, 26% and 11% of participants, respectively. Oncogenic HPV infections were more likely to persist than non-oncogenic infections for ≥ 6, 12 and 18 months or longer (61% vs. 27%, p = 0.006; 45% vs. 19%, p = 0.0279; and 34% vs. 12%; p = 0.0422, respectively); whereas the likelihood of 24-month persistent infections was similar between oncogenic and non-oncogenic infections (11% vs. 12%; p = 0.982). The two most common oncogenic HPV types that persisted for ≥ 24 months were HPV-18 (50%) and HPV-51 (15%); the two most common non-oncogenic types were HPV-11 (25%) and HPV-6 (22%). When examined by sample type, there were no statistically significant differences in any HPV persistence by anatomical site/fluid at ≥ 6, 12, 18 and 24 months.

Figure 1 (A-D) describes durations of persistence for any, oncogenic and non-oncogenic HPV infection detected in any sample, penile, urethral and semen samples. The median duration of persistence for any HPV at any sampling site was 21.7 months (95% CI: 19.0-24.4), with 36% of infections remaining persistent at 24 months after initial HPV detection. Median durations were not statistically different between oncogenic persistence (22.5 months; 95% CI: 19.1-25.9) and non-oncogenic persistence (18.4 months; 95% CI: 14.1-22.7). While the median duration of persistence appeared to be longer for

Table I. Baseline characteristics of men (n = 50), Palermo, Italy, 2006-2009.

Variable	n (%)
Age, years	
20-34	31 (62.0)
35-60	19 (38.0)
Age at sexual debut	
≤ 16	19 (38.0)
17-20	30 (60.0)
> 20	1 (2.0)
Current sexual partner	
No	9 (18.0)
Yes	41 (82.0)
Duration of current relationship, years	
No relationship	9 (18.0)
≤ 1	13 (26.0)
> 1	28 (56.0)
Lifetime number of sexual partners	
≤ 5	14 (28.0)
> 5	36 (72.0)
Recent risky sexual behaviour[a]	
No	38 (76.0)
Yes	12 (24.0)
Condom use	
Never	11 (22.0)
Sometimes	30 (60.0)
Always	9 (18.0)
Engage in oral sex	
No	10 (20.0)
Yes	40 (80.0)
History of sexually transmitted infection	
No	38 (76.0)
Yes	12 (24.0)
Presence of external genital warts	
No	43 (86.0)
Yes	7 (14.0)
Presence of external genital warts in sexual partner	
No	45 (90.0)
Yes	5 (10.0)
Confirmed HPV infection in sexual partner	
No	7 (14.0)
Yes	43 (86.0)
Smoking status	
Never	17 (34.0)
Former	4 (8.0)
Current	29 (58.0)
Current alcohol use	
No	13 (26.0)
Yes	37 (74.0)

[a]Defined as having sexual intercourse with a casual acquaintance without condoms, while under the influence of alcohol/drugs.

Table II. Percent of participants with 6-, 12-, 18- or 24-month persistent HPV infections with any, oncogenic and non-oncogenic HPV based on type-specific analysis.

	Any site, n (%)				Penis, n (%)				Urethra, n (%)				Semen, n (%)			
	6-mo[a]	12-mo[b]	18-mo[c]	24-mo[d]	6-mo[a]	12-mo[b]	18-mo[c]	24-mo[d]	6-mo[a]	12-mo[b]	18-mo[c]	24-mo[d]	6-mo[a]	12-mo[b]	18-mo[c]	24-mo[d]
Any HPV persistence	34 (49)	25 (36)	18 (26)	8 (11)	29 (62)	19 (40)	15 (32)	5 (11)	18 (72)	11 (44)	7 (28)	2 (8)	19 (59)	8 (25)	3 (9)	1 (3)
Oncogenic HPV persistencee	27 (61)	20 (45)	15 (34)	5 (11)	21 (55)	15 (39)	12 (32)	3 (8)	12 (63)	7 (37)	6 (32)	1 (5)	13 (52)	7 (28)	3 (12)	1 (4)
Single oncogenic HPV	14 (78)	13 (72)	11 (61)	2 (11)	13 (62)	11 (52)	10 (48)	2 (10)	8 (100)	4 (50)	5 (63)	1 (13)	10 (83)	6 (50)	3 (25)	1 (8)
Multiple oncogenic HPV	13 (50)	7 (27)	4 (15)	3 (12)	8 (47)	4 (24)	2 (12)	1 (6)	4 (36)	3 (27)	1 (9)	–	3 (23)	1 (8)	–	–
16	7 (64)	5 (45)	4 (36)	1 (9)	6 (60)	4 (40)	3 (30)	1 (10)	3 (100)	1 (33)	1 (33)	–	5 (71)	3 (43)	2 (29)	–
18	3 (75)	3 (75)	3 (75)	2 (50)	3 (75)	2 (50)	2 (50)	1 (25)	–	–	–	–	2 (100)	2 (100)	1 (50)	1 (50)
31	6 (35)	2 (12)	2 (12)	–	4 (29)	2 (14)	2 (14)	–	–	–	–	–	1 (50)	–	–	–
33	1 (25)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
51	9 (69)	7 (54)	5 (38)	2 (15)	5 (50)	3 (30)	3 (30)	1 (10)	4 (67)	3 (50)	2 (33)	–	4 (44)	2 (22)	–	–
52	1 (25)	1 (25)	–	–	1 (100)	1 (100)	–	–	–	–	–	–	–	–	–	–
53	4 (44)	3 (33)	2 (22)	1 (11)	2 (33)	1 (17)	1 (17)	–	3 (75)	3 (75)	1 (25)	1 (25)	–	–	–	–
56	2 (40)	1 (20)	–	–	2 (50)	1 (25)	–	–	–	–	–	–	–	–	–	–
58	3 (50)	1 (17)	–	–	2 (33)	1 (17)	–	–	1 (100)	–	–	–	–	–	–	–
59	1 (50)	1 (50)	1 (50)	–	1 (100)	1 (100)	1 (100)	–	1 (100)	1 (100)	1 (100)	–	1 (50)	–	–	–
66	8 (57)	4 (29)	3 (21)	2 (14)	6 (46)	3 (23)	2 (15)	1 (8)	4 (57)	2 (29)	2 (29)	–	3 (43)	1 (14)	–	–
68	2 (40)	1 (20)	–	–	1 (25)	–	–	–	1 (100)	–	–	–	–	–	–	–
Non-oncogenic HPV persistence[f]	7 (27)	5 (19)	3 (12)	3 (12)	8 (89)	4 (44)	3 (33)	2 (22)	6 (100)	4 (67)	1 (17)	1 (17)	6 (86)	1 (14)	–	–
6	8 (89)	6 (67)	3 (33)	2 (22)	8 (89)	5 (56)	3 (33)	2 (22)	5 (83)	2 (33)	1 (17)	–	4 (67)	1 (17)	–	–
11	2 (50)	2 (50)	1 (25)	1 (25)	2 (50)	2 (50)	1 (25)	–	1 (100)	1 (100)	–	–	–	–	–	–
42	2 (67)	2 (67)	–	–	1 (50)	1 (50)	–	–	1 (100)	1 (100)	–	–	–	–	–	–
43	2 (67)	1 (33)	–	–	1 (50)	1 (50)	–	–	1 (50)	–	–	–	1 (50)	–	–	–
44	1 (17)	1 (17)	–	–	–	–	–	–	1 (100)	1 (100)	–	–	–	–	–	–
54	2 (50)	1 (25)	–	–	2 (50)	1 (25)	–	–	2 (100)	–	–	–	–	–	–	–
62	3 (60)	2 (40)	2 (40)	1 (20)	3 (100)	2 (67)	2 (67)	1 (33)	2 (100)	1 (50)	1 (50)	1 (50)	1 (25)	–	–	–
84	1 (50)	–	–	–	–	–	–	–	–	–	–	–	1 (50)	–	–	–
91	1 (100)	–	–	–	1 (100)	–	–	–	–	–	–	–	–	–	–	–

Note: Men with 12-month persistence were also counted in 6-month persistence; men with 18-month persistence were counted in 6- and 12-month persistence; men with 24-month persistence were counted in 6-, 12- and 18-month persistence. [a]6-month persistent infection was defined as the detection of same HPV genotypes at the same or any infection site at ≥ 2 consecutive visits. [b]12-month persistent infection was defined as the detection of same HPV genotypes at the same or any infection site at ≥ 3 consecutive visits. [c]18-month persistent infection was defined as the detection of same HPV genotypes at the same or any infection site at ≥ 4 consecutive visits. [d]24-month persistent infection was defined as the detection of same HPV genotypes at the same or any infection site at 5 consecutive visits. [e]Defined as 6-, 12- or 18-month persistent infection with any oncogenic HPV types regardless of the co-infection with non-oncogenic types. [f]Defined as 6-, 12- or 18-month persistent infection with non-oncogenic HPV types only.

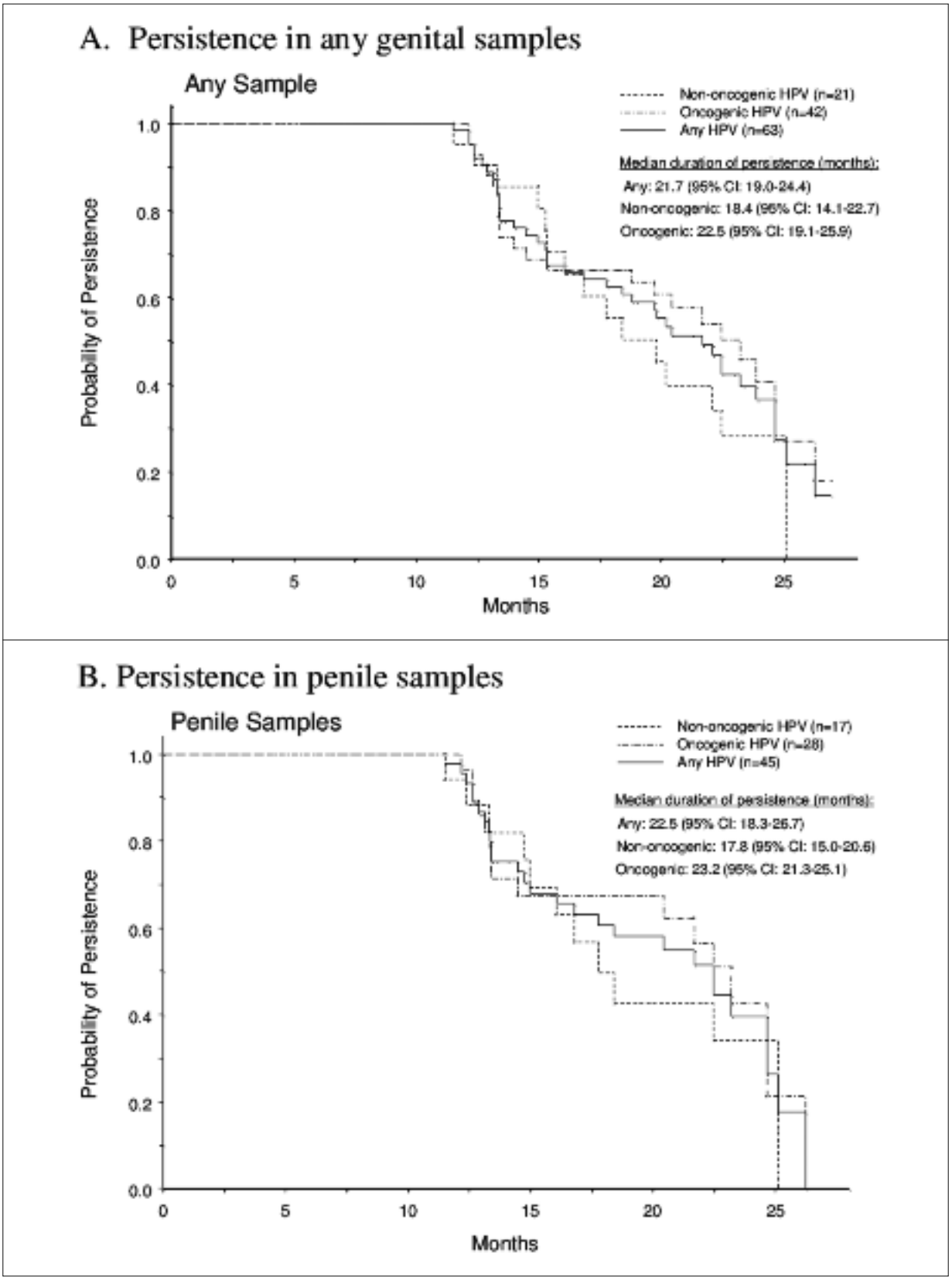

Figure 1. *Continued*

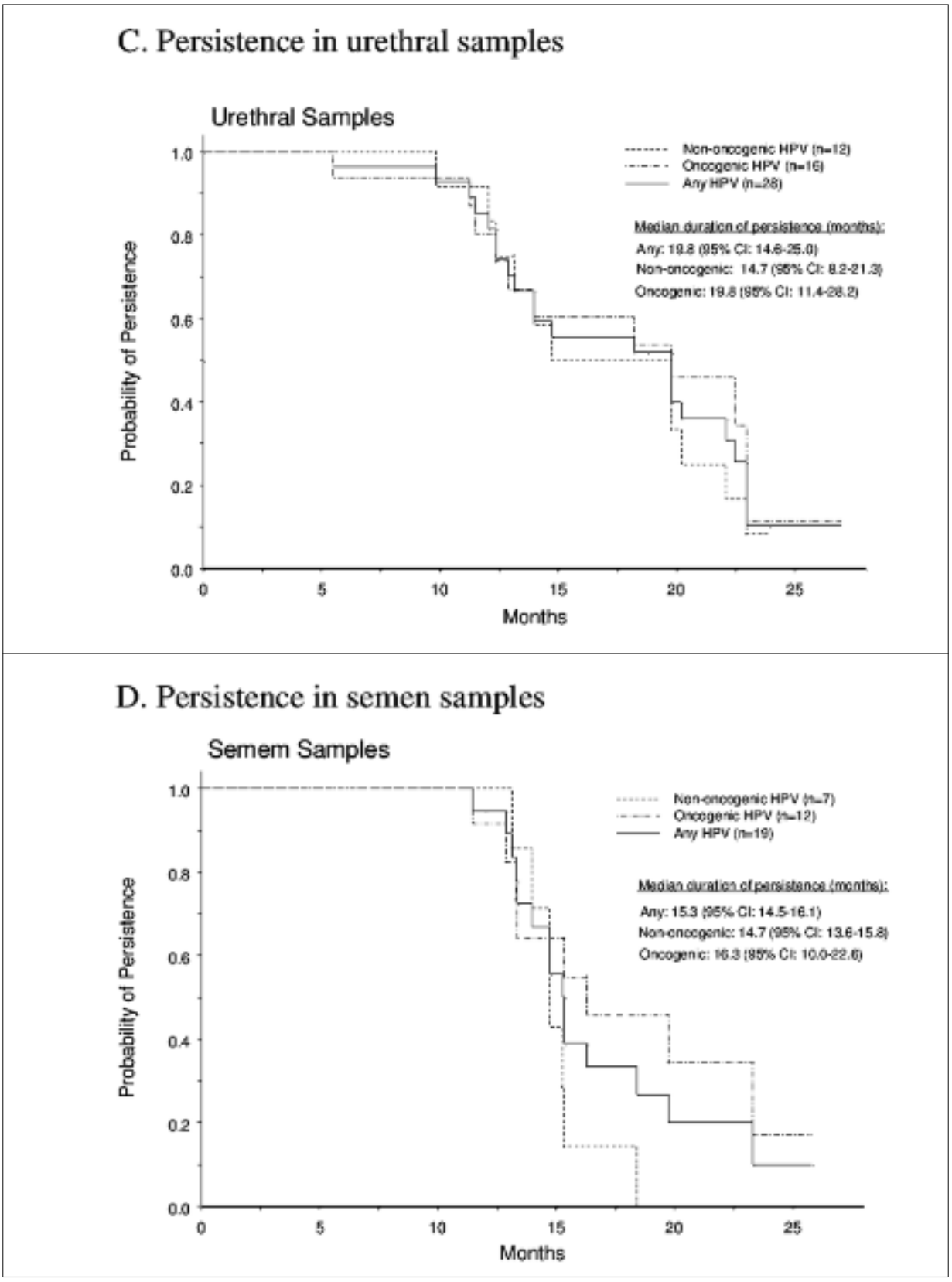

Figure 1. Duration of persistence for any, oncogenic and non-oncogenic HPV infection detected in any and individual genital samples.

penile infections than infections at any other site, a statistically significant difference was only observed in the median duration of any HPV persistence between penile samples (22.5 months, 95% CI: 18.3-26.7) and semen samples (15.3 months; 95% CI: 14.5-16.1).

With all genital samples combined, socio-behavioural factors were not associated with type-specific persistence of any HPV at ≥ 6, 12, 18, or 24 months (data not shown).

Discussion

This prospective cohort study investigated HPV persistence among men with a prevalent genital HPV infection detected at the baseline, over a 2-year study period. Taking into account the difficulty in confirming HPV clearance status in an HPV natural history study, negative status after having baseline HPV positive status was best defined in this study by the absence of HPV DNA detection in all of three genital sites examined, namely, penile surface, urethra and semen. On the other hand, a positive HPV status was ascertained by employing a combined use of two highly sensitive molecular HPV assays, to assess the full spectrum of infecting types and low-level HPV infections[18,19].

Genital HPV persistence is common among this group of men. With all genital samples combined, 49% of men had a genital HPV infection that lasted at least 6 months, while infection in 36%, 26% and 11% of men persisted for 12, 18 and 24 months or longer, respectively.

Only a few epidemiological studies on HPV persistence in men included DNA samples from multiple anatomic sites. Giuliano et al[17,21] employed HPV sampling and DNA detection methods similar to those used in the present analysis, and reported comparable persistence rates with 6-month persistence observed in 44.5% of initial infection, 12-month persistence in 25.2% and 18-month persistence in 10.7% of initial infection. A slightly lower rate of 6-month persistence was reported in Danish soldiers by Kjaer et al[10]. Despite differences in sampling protocols, participant demographic or behavioural profiles or the use of HPV DNA detection methods with different sensitivity between studies, various studies have shown that a 6-month persistent genital infection is very common among men. Given that the majority of sexual partners of study participants had confirmed HPV infection at the study baseline, it is difficult to determine the original source of infection within the relationship. However, persistent infection among study participants, regardless of anatomical sites that harbour the virus, will undoubtedly increase the likelihood of future transmission to their sexual partners and impact on their partners' risk of HPV-associated diseases. This finding supports the public health importance of preventing HPV infection in men as a potential source of infection for their sexual partners using effective measures such as HPV vaccination.

This study found that oncogenic genital HPV infection was more likely to persist for 6 months or longer than non-oncogenic genital HPV infection (61% and 27%, respectively), whereas the likelihood of 24-month persistence was similar between oncogenic and non-oncogenic infection (11% and 12%, respectively). This finding was consistent with that of Giuliano et al[13], who found that rates of 6-, 12- and 18-month persistent infections were 41.7%, 19.0% and 0% for oncogenic HPV types, respectively, compared to 46.5%, 29.3% and 14.4%, respectively, for non-oncogenic types. This aspect of genital HPV infection in men is different from what has been shown in cervical HPV infection in women, which is characterised by a longer duration of infection with oncogenic HPV types, compared with non-oncogenic types[22].

Sampling multiple anatomic sites in men is considered as the best sampling strategy[14,16] to increase the likelihood of HPV DNA detection in the male external genital area. However, we found that the rates of persistence for any HPV or individual HPV type were comparable between three anatomic sites that we sampled, although more HPV genotypes were detected in penile samples than urethral or seminal samples. Thus, our data suggest that sampling of multiple genital sites may not be required for measuring the persistence of HPV infection in men, and any of the three genital samples (penile, urethral or semen sample) could be used for assessing duration of genital HPV persistence.

When combining any genital site and any HPV genotype, a mean duration of 21.7-month persistence was observed in this study. This value is different from that reported in other studies, which is in the range 5.9-7.5 months[13,23]. One likely explanation is the fact that approximately 86% of the study cohort consisted of male partners of women with cervical HPV infection, who might have contributed to the HPV (re) infection of their male partners.

When examining which genital site was more prone to a longer duration of infection, an increased duration was observed in penile samples (22.5 months) compared to seminal samples (15.3 months). The presence of HPV DNA in semen for more than one year merits particular attention. Recent studies[24,25] reported HPV DNA presence in spermatozoa, Sertoli and Leydig cells[26] and the vas deferens[27], leading to a hypothesized role for HPV infection in male infertility[16,24] as well as unfavourable reproductive outcomes in infertile couples undergoing *in vitro* fertilization procedures[28].

The lack of association of duration of persistence with socio-behavioural factors in men was in agreement with findings from a previous study[13]. Circumcision seems to be associated with reduced persistence in men[9,29,30], even though the mechanism of protection is unclear. Since this practice is not common in our geographical area and then in our study sample, the effect of circumcision on the duration of HPV infection could not be examined in this study. Further research is required to determine whether long-term HPV infection is associated with impairment in host immune responses or heterogeneity in virus-host interactions, as well as to evaluate the important role of the sexual partner in the viral dynamics of genital HPV infection in men.

This study's unique place in the literature is that the majority of participants were in a relationship with a female with confirmed cervical HPV infection, and therefore the study documents persistence at the penis/urethra/semen in men who are at high risk for new HPV infections. A limitation of this report is that the cohort was derived from a highly selective population, and the estimates of persistence are not representative of the general population and may not be generalizable. Moreover, the small sample size of this paper limited our ability to perform further analysis on persistence of individual HPV types, such as assessing the duration of persistence and the impact of co-infection on the duration of persistence.

In 2006, the US Food and Drug Administration approved the first prophylactic HPV vaccine, to be used for young women before sexual debut; in 2011 it approved the use of HPV vaccines for anal cancer prevention in men.

Conclusions

Since HPV vaccination is gaining increasing attention as a potential preventive approach also for men, it is imperative to obtain additional insight from studies on natural history of HPV infection in men, particularly as far as incidence and duration are concerned.

Conflict of Interest

The Authors have no COI, including financial interests or connections, direct or indirect, or any other situations that could raise questions of bias in either the reported work or the conclusions, implications, or opinions stated.

References

1) Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, Hillman RJ, Petoumenos K, Roberts J, Tabrizi SN, Templeton DJ, Grulich AE. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic reviewand meta-analysis. Lancet Oncol 2012; 13: 487-500.

2) Ting J, Rositch AF, Taylor SM, Rahangdale L, Soeters HM, Sun X, Smith JS. Worldwide incidence of cervical lesions: a systematic review. Epidemiol Infect 2015; 143: 225-241.

3) De Villiers EM, Fauquet C, Broker T, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology 2004; 324: 17-27.

4) Castle PE, Rodríguez AC, Burk RD, Herrero R, Wacholder S, Alfaro M, Morales J, Guillen D, Sherman ME, Solomon D, Schiffman M; Proyecto Epidemiológico Guanacaste (PEG) Group. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. Br Med J 2009; 339: 1-13.

5) Koshiol J, Lindsay L, Pimenta J, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008; 168: 123-137.

6) Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V; WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--part b: biological agents. Lancet Oncol 2009; 10: 321-322.

7) Castellsagué X, Bosch FX, Muñoz N, Meijer CJ, Shah KV, de Sanjose S, Eluf-Neto J, Ngelangel CA, Chichareon S, Smith JS, Herrero R, Moreno V, Franceschi S; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 2002; 346: 1105-1112.

8) Van Doornum GJ, Prins M, Juffermans LH, Hooykaas C, van den Hoek JA, Coutinho RA, Quint WG. Regional distribution and incidence of human papillomavirus infections among heterosexual men and women with multiple sexual partners: a prospective study. Genitourin Med 1994; 70: 240-246.

9) Wikström A, Popescu C, Forslund O. Asymptomatic penile HPV infection: a prospective study. Int J STD AIDS 2000; 11: 80-84.

10) Kjaer SK, Munk C, Falck Winther J, Jørgensen HO, Meijer CJ, van den Brule AJ. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. Cancer Epidemiol Biomarkers Prev 2005; 14: 1528-1533.

11) Lajous M, Mueller N, Cruz-Valdéz A, Aguilar LV, Franceschi S, Hernández-Avila M, Lazcano-Ponce E. Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. Cancer Epidemiol Biomarkers Prev 2005; 14: 1710-1716.

12) Partridge JM, Hughes JP, Feng Q, Winer RL, Weaver BA, Xi LF, Stern ME, Lee SK, O'Reilly SF, Hawes SE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. J Infect Dis 2007; 196: 1128-1136.

13) Giuliano AR, Lu B, Nielson CM, Flores R, Papenfuss MR, Lee JH, Abrahamsen M, Harris RB. Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. J Infect Dis 2008; 198: 827-835.

14) Giovannelli L, Migliore MC, Capra G, Caleca MP, Bellavia C, Perino A, Viviano E, Matranga D, Ammatuna P. Penile, urethral, and seminal sampling for diagnosis of human papillomavirus infection in men. J Clin Microbiol 2007; 45: 248-251.

15) Weaver BA, Feng Q, Holmes KK, Kiviat N, Lee SK, Meyer C, Stern M, Koutsky LA. evaluation of genital sites and sampling techniques for detection of human papillomavirus DNA in men. J Infect Dis 2004; 189: 677-685.

16) Nielson CM, Flores R, Harris RB, Abrahamsen M, Papenfuss MR, Dunne EF, Markowitz LE, Giuliano AR. human papillomavirus prevalence and type distribution in male anogenital sites and semen. Cancer Epidemiol Biomarkers Prev 2007; 16: 1107-1114.

17) Giuliano AR, Lazcano-Ponce E, Villa LL, Flores R, Salmeron J, Lee JH, Papenfuss MR, Abrahamsen M, Jolles E, Nielson CM, Baggio ML, Silva R, Quiterio M. the human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. Cancer Epidemiol Biomarkers Prev 2008; 17: 2036-2043.

18) Giovannelli L, Lama A, Capra G, Giordano V, Arcò P, Ammatuna P. Detection of human papillomavirus DNA in cervical samples: analysis of the new PGMY-PCR compared to the hybrid Capture II and MY-PCR assays and a two-step Nested PCR assay. J Clin Microbiol 2004; 42: 3861-3864.

19) Harnish DG, Belland LM, Scheid EE, Rohan TE. evaluation of human papillomavirus-consensus primers for hpv detection by the polymerase chain reaction. Mol Cell Probes 1999; 13: 9-21.

20) Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, Lindeman J, ter Harmsel B, Burger M, Quint W. development and clinical evaluation of a highly sensitive pcr-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999; 37: 2508-2517.

21) Giuliano AR, Nyitray AG, Kreimer AR, Pierce Campbell CM, Goodman MT, Sudenga SL, Monsonego J, Franceschi S. Differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. J Cancer 2015; 136: 2752-2760.

22) Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Désy M, Rohan TE. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999; 180: 1415-1423.

23) Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, Abrahamsen M, Salmeron J, Anic GM, Rollison DE, Smith D. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet 2011; 12: 932-940.

24) Kaspersen MD, Larsen PB, Ingerslev HJ, Fedder J, Petersen GB, Bonde J, Höllsberg P. Identification of multiple HPV types on spermatozoa from human sperm donors. PLoS One 2011; 6: 18095-18099.

25) Schillaci R, Capra G, Bellavia C, Ruvolo G, Scazzone C, Venezia R, Perino A. Detection of oncogenic human papillomavirus genotypes on spermatozoa from male partners of infertile couples. Fertil Steril 2013; 100: 1236-1240.

26) Martorell M, Gil-Salom M, Pérez-Vallés A, Garcia JA, Rausell N, Senpere A. Presence of human papillomavirus DNA in testicular biopsies from nonobstructive azoospermic men. Arch Pathol Lab Med 2005; 129: 1132-1136.

27) Rintala MA, Pöllänen PP, Nikkanen VP, Grénman SE, Syrjänen SM. Human papillomavirus DNA is found in the vas deferens. J Infect Dis 2002; 185: 1664-1667.

28) Perino A, Giovannelli L, Schillaci R, Ruvolo G, Fiorentino FP, Alimondi P, Cefalù E, Ammatuna P. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. Fertil Steril 2011; 95: 1845-1848.

29) Castellsagué X, Bosch FX, Muñoz N, Meijer CJ, Shah KV, de Sanjose S, Eluf-Neto J, Ngelangel CA,

Chichareon S, Smith JS, Herrero R, Moreno V, Franceschi S; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 2002; 346: 1105-1112.

30) Lu B, Wu Y, Nielson CM, Flores R, Abrahamsen M, Papenfuss M, Harris RB, Giuliano AR. Factors associated with acquisition and clearance of human papillomavirus infection in a cohort of US men: a prospective study. J Infect Dis 2009; 199: 362-371.