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Original article

Natural History of Multiple Human Papillomavirus Infections in Female Adolescents With Prolonged Follow-up

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A B S T R A C T

Purpose: The aim of the study was to better characterize the natural history of human papillomavirus (HPV) infections in female adolescents.
Methods: Female adolescents were enrolled in a longitudinal study. Self-vaginal samples were obtained every 3 months and tested for HPV. No participants received HPV vaccination. The findings for 40 female adolescents with the longest follow-up are reported in this study.
Results: Average age at the time of enrollment was 15.2 years (range: 14–17; SD: .97). Mean duration of follow-up was 6.7 years (range: 4.4–9.2; SD: 1.2). In all, 32 participants (80%) reported being involved in sexual activity before their enrollment in the study; all reported being involved in sexual activity before enrollment; all reported being involved in sexual activity during follow-up. Baseline and cumulative prevalence of HPV among participants was 55% and 100%, respectively. During the study, each participant tested positive for a mean of 14 HPV types. Cumulatively, HPV 16 was detected in 29 of 40 participants (72.5%). Mean duration of high- and low-risk infections was 655.9 (median: 433) and 524.1 days (median: 334), respectively.
Conclusion: With prolonged follow-up, HPV infections with multiple types were found in all participants. Most had infection with HPV-16 or HPV-18, the oncogenic types represented in current vaccines, as well as infection with other oncogenic types. These data reinforce the importance of vaccine and non-vaccine strategies for prevention of HPV infections.

Human papillomavirus (HPV) has been reported to be the most commonly acquired sexually transmitted infection (STI) in the United States [1]. Given that the incidence of initial genital HPV infections reaches the maximum shortly after sexual debut, efforts to fully elucidate the natural history of HPV in adolescents are important in targeting key public health interventions [2,3]. However, complete understanding is made complex by the presence of more than 40 types of HPV known to infect the genital epithelia. Our understanding of HPV pathogenesis has progressed tremendously in recent years, leading to the development of prophylactic vaccines to prevent infections by two specific HPV types causally associated with most cervical and other anogenital cancers ("high risk," or high-risk [HR] types), and two HPV types associated with genital warts and low-grade cervical dysplasia ("low risk," or low-risk [LR] types) [4,5]. However, enthusiasm over this public health success is tempered by realities regarding the availability and uptake of HPV vaccine: only a moderate fraction of eligible girls and women worldwide have received HPV vaccination or are likely to be vaccinated in the near future [6]. Although the current HPV vaccines protect against the most common disease-causing types, vaccinated individ-

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uals remain vulnerable to infection and cytologic abnormalities due to non-vaccine types. These reasons emphasize the need for ongoing development of HPV prevention and treatment strategies that include, but are not limited to, HPV vaccination.

To address the needs of prevention programs, more extensive characterization of HPV infections from the time of acquisition to adulthood is needed. However, few studies have longitudinally examined HPV infections in female adolescents. Most infections in adolescents and young women are thought to have a limited duration, and the role of additional type-specific infections or reactivation of previous infections that become undetected by current methods remains poorly understood. The widespread presence of a variety of HPV types increases the likelihood that multiple HPV infections—rather than a single HPV infection—characterize the natural history of HPV infections [7–12].

In a previous study with shorter follow-up, 60 female adolescents were followed up for an average of 2.2 years [13]. The cumulative prevalence of HPV was >80%; 40 of 49 HPV-positive female adolescents (81.6%) were found to be positive for multiple HPV types. In the current study, 40 unvaccinated female adolescents were evaluated for a longer time to describe the natural history of HPV infection. Self-collected vaginal samples were frequently obtained from the participants and sensitive HPV detection and genotyping methods were used. This investigation generated a set of intensive HPV testing records for a prolonged period. The data were used to better characterize HPV natural history in a group of urban young women during the transitional period between childhood and young adulthood.

Methods

Participants

A longitudinal study of STI and sexual behaviors in a cohort of 120 female adolescents was recently completed [13,14]. In the present study, data from 40 adolescents with the longest duration of follow-up were analyzed for HPV infections. Participants were enrolled under the main study protocol, which was approved by the local institutional review board. Inner-city female adolescents attending one of the three primary care clinics in Indianapolis, IN, were eligible for enrollment in this study. Inclusion criteria for the comprehensive study and this analysis were as follows: age 14–17 years, able to understand English and provide written consent, not have any serious psychiatric problems or mental deficiencies, and have one parent who was able to give permission for participation in the study. For this study, sexual activity was defined as vaginal intercourse, because this definition was clear to both the participants and to the investigative team. Sexually active and inactive adolescents were enrolled; however, adolescents who were pregnant at the time of enrollment were excluded. Participant informed consent and parental consent were obtained at the time of enrollment. All participants received financial compensation for their time and effort.

Sample collection

Participants were evaluated every 3 months. A self-collected vaginal sample for STI testing was obtained, and a face-to-face interview regarding sexual behavior was completed. During alternating 3-month periods between study visits, participants collected weekly self-collected vaginal samples for other STI studies. Vaginal swab samples are of interest because women can easily perform the sampling, and HPV testing of self-vaginal swabs approximates that of clinician-obtained cervical swabs [13,15–21]. In addition, our prior longitudinal work indicated that vaginal swabs accurately reflected the HPV type distribution of that in clinician obtained vaginal swabs [13].

Dry vaginal samples were transported to the laboratory, and then vortexed in a tube containing 1 mL of sterile water. The water was subsequently stored at −20°C until processing for HPV testing. If a quarterly vaginal sample was unavailable for testing, a weekly vaginal sample collected within 14 days before or after the quarterly sample collection date was substituted.

HPV detection and genotyping

Deoxyribonucleic acid (DNA) was extracted from samples, as described previously [22,23]. The Linear Array HPV Genotyping Test (LA-HPV; Roche Molecular Diagnostics, Indianapolis, IN) was used for HPV detection and genotyping [13,22,23]. This assay detects 37 types of HPV infection using nondegenerate, 5′-biotin-labeled primer pools for polymerase chain reaction amplification within the L1 region of the HPV genome. Reactions were amplified in a PerkinElmer TC9600 Thermal Cycler (PerkinElmer, MA), as described previously [24,25]. A positive control reaction (provided by Qiagen) and a negative control reaction (no DNA) were performed with each assay. The GH20/PC04 human β-globin target was co-amplified to determine sample adequacy.

Detection of specific HPV types was performed as described previously [13,26]. The LA-HPV is an expanded version of a previous Roche line blot assay used in our prior longitudinal analysis. The 37 individual HPV types detected in the assay are 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, and IS39 (HR), and 6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, and CP6108 (LR).

Most samples in the current study had been tested before using the previous LA-HPV assay, which detected 27 types of HPV infection. All samples were retested using the newer LA-HPV, and only these results were used in this analysis.

Statistical methods

Two separate analyses were performed: one at the sample level and one at the participant level. The distribution of HPV type was analyzed at the sample level. At the participant level, baseline and end-of-study prevalence rates, as well as cumulative prevalence rates of type-specific HPV infections, were calculated and reported. A participant was considered to have a type-specific HPV infection if one or more quarterly samples tested positive for that HPV type during the study. For an individual participant, subsequent samples tested positive for an HPV type previously detected were considered part of the original infection. The duration of an infection was defined as the time between initial detection and last detection of an HPV type within the individual. If a type-specific infection persisted until the end of the observation period, the duration of the infection was considered as censored, meaning that the infection lasted at least until the end of observation.

Summary statistics were calculated for the categories of HR, LR, and all types of HPV infection. Median durations of HR and LR HPV infections were compared using proportional hazard re-

gression models [27]. To accommodate the within-participant correlation among the infection episodes contributed by the same participant, we included a random participant effect in the proportional hazard model [27]. All analysis was performed using SAS 9.1.3 (SAS Institute, Inc., Cary, NC) [28].

Results

Demographics

Specific characteristics of the study participants are summarized in Table 1. The mean duration of follow-up was 6.7 years (range: 4.4–9.2; SD: 1.2). None of the participants received HPV vaccination during the study period (1999–2008) because the vaccine was not yet available in the clinics.

Sample level analysis of HPV: Distribution of HPV types

Self-collected vaginal samples (n = 1,015) were analyzed (mean: 25.5 samples/participant; range: 8–35; SD: 5.1). Of all samples tested, 96.8% (n = 982) yielded a positive result for human β-globin in the LA-HPV and were included in the HPV analysis. Overall, HPV was detected in 739 of samples (75.3%). The HR types were detected in 647 samples (65.9%) and LR types in 453 samples (46.1%).

HPV-16 was the most frequently detected HR type, found in 14.4% of the samples, followed by HPV-52 which was found in 11.1% of the samples (Table 2). HPV-62 was the most frequently found LR type (11.8% of samples) (Table 2).

Frequent detection of multiple HPV types in samples

An average of 2.12 different HPV types was detected per β-globin-positive sample (range: 0–11; SD: 2.03). A single HPV type was detected in 23.6% of the samples, and ≥2 types were detected in 51.6% of the samples.

Participant level analysis: Prevalence of HPV

Enrollment and final sample results per participant were compared with the results for the entire study period. At enrollment, 55% of participants were HPV positive; at the last visit, 78% were HPV positive. Detection of both HR and LR HPV types increased during the study (Table 3). All the participants (100%) were infected with at least 1 HR HPV type and 100% of participants were infected with at least one LR HPV type at some point over the entire study period.

We found a high cumulative prevalence of HPV-16, detected at some point in 72.5% of participants, making it the most frequently detected HR type (Table 4), followed by HPV-59 which was found in 65% of the participants (Table 4). HPV-6 and HPV-62 were the most frequently detected LR types (62.5% of participants) (Table 4). Vaccine HPV types (6, 11, 16, or 18) were detected at some point in the study in 92.5% of participants (N = 37) (Table 5). Fourteen participants (35%) were found to be infected with both HPV-16 and HPV-18. Figure 1 depicts the patterns of

Table 1
Participant characteristics as reported at enrollment visit (n = 40)

Characteristic	Number (%) or mean ± SD
Age (years)	15.2 ± .97
Race	
African American	38 (95%)
Caucasian	2 (5%)
Sexual experience (ever had sex)	
At enrollment	32 (80%)
At study end	40 (100%)
Age at the time of first sex (N = 32)[a]	13.8 ± 1.12
Number of sexual partners[a]	
Within past 2 months	1.97 ± .86
In lifetime	3.8 ± 3.35
History of STI (N = 39)[b]	15 (38.5%)

[a] Includes only those participants who reported being sexually active at the time of or before enrollment.
[b] Self-reported history of gonorrhea, chlamydia, or trichomonas.

Table 2
Distribution of high-risk (HR) and low-risk (LR) HPV types among genital samples with positive β globin

HPV types	Samples (N = 982)	%
HR type		
HPV 16	141	14.4
HPV 18	75	7.6
HPV 26	6	0.6
HPV 31	49	5.0
HPV 33	27	2.8
HPV 35	77	7.8
HPV 39	66	6.7
HPV 45	56	5.7
HPV 51	97	9.9
HPV 52	109	11.1
HPV 53	86	8.8
HPV 56	48	4.9
HPV 58	85	8.7
HPV 59	98	10.0
HPV 66	83	8.5
HPV 67	39	4.0
HPV 68	83	8.5
HPV 69	6	0.6
HPV 70	48	4.9
HPV 73	40	4.1
HPV 82	16	1.6
IS39	2	0.2
LR type		
HPV 6	62	6.3
HPV 11	14	1.4
HPV 40	37	3.8
HPV 42	52	5.3
HPV 52	64	6.5
HPV 55	68	6.9
HPV 61	64	6.5
HPV 62	116	11.8
HPV 64	3	0.3
HPV 71	2	0.2
HPV 72	13	1.3
HPV 81	38	3.9
HPV 83	81	8.3
HPV 84	71	7.2
CP6108	60	6.1

Table 3
HPV prevalence at the time of study enrollment, at final study visit, and cumulatively (N = 40)

	Any HPV N (%)	High-risk HPV N (%)	Low-risk HPV N (%)
Enrollment visit	22 (55)	18 (45)	12 (30)
Final study visit	31 (78)	26 (65)	22 (55)
Cumulative	40 (100)	40 (100)	40 (100)
Cumulative[a]	40 (100)	40 (100)	39 (97.5)

[a] Excluding single positive samples.

Table 4
Distribution of high-risk (HR) and low-risk (LR) HPV types among participants

HPV types	Participants (N = 40)	%
HR type		
HPV 16	29	72.5
HPV 18	18	45
HPV 26	4	10
HPV 31	12	30
HPV 33	4	10
HPV 35	21	52.5
HPV 39	17	42.5
HPV 45	15	37.5
HPV 51	25	62.5
HPV 52	20	50
HPV 53	19	47.5
HPV 56	15	37.5
HPV 58	17	42.5
HPV 59	26	65
HPV 66	24	60
HPV 67	20	50
HPV 68	15	37.5
HPV 69	1	2.5
HPV 70	6	15
HPV 73	11	27.5
HPV 82	8	20
IS39	1	2.5
LR type		
HPV 6	25	62.5
HPV 11	9	22.5
HPV 40	17	42.5
HPV 42	20	50
HPV 52	19	47.5
HPV 55	16	40
HPV 61	17	42.5
HPV 62	25	62.5
HPV 64	2	5
HPV 71	2	5
HPV 72	6	15
HPV 81	12	30
HPV 83	19	47.5
HPV 84	24	60
CP6108	19	47.5

HPV infection for three selected participants during the study period. Panel A represents a participant infected with few HPV types, predominantly in the second half of the study participation. HR types detected were HPV-16, -51, and -82; LR types included HPV-6, -40, and -54. Panel B represents a participant with eight HR types and seven LR types of HPV infection. Many of these infections were overlapping and were concentrated in years 2–4 of the participation period. The participant in panel C had a large number of type-specific infections and the enrollment sample tested positive for five HPV types, including 16 and 18 (HR) and 42, 62, and 84 (LR). None of this participant's samples were negative. At any one point, she was infected with 1–8 types.

Each of the selected participants in Figure 1 has at least one HPV type-specific infection that demonstrated the following pattern: detection of HPV followed by a period of nondetection of ≥6 months (defined as two or more consecutive negative tests), then redetection of the same HPV type (panel A: HPV-16, panel B: HPV-51, panel C: HPV-39, -51, -52, -56, -66, -62, -81, -84, and CP1608). A total of 31 participants had 100 infections (67 HR and 33 LR) that were characterized by such a pattern. This represented 17.9% of all type-specific infections among all participants.

Frequent detection of multiple HPV types in female adolescents

Over the entire study period, participants on average tested positive for 14 type-specific HPV infections (range: 4–24; SD: 5.11). Each participant had a mean of 8.2 HR types (range: 2–14; SD: 3.16) and 5.8 LR types (range: 1–11; SD: 2.4) detected at some point during the study. Participants had a mean of 2 vaccine types (range: 0–4; SD: .95).

The samples collected at the time of enrollment and at the end of the study for each participant were analyzed for number of type-specific infections present, and were compared with similar cumulative data. Eighteen participants did not have HPV infection at the time of enrollment. The enrollment samples of the participants with HPV had a mean 2.6 HPV types per participant (range: 1–7; SD: 1.9). Participants had a mean 1.6 HR types (range: 0–5; SD: 1.3) and a mean 1 LR types (range: 0–4; SD: 1.1) at the time of enrollment. At their final visit, nine participants had no HPV infection detected. Participants had a mean of 2.4 HPV types (range: 1–7; SD: 1.4), a mean 1.4 HR types (range: 0–5; SD: 1.1), and a mean 1 LR types (range: 0–3; SD: .8) at the end of the study.

Additional analysis excluding single positive samples

Detection of HPV in only one sample from a participant is defined as a "single positive," which may represent true infection, HPV viral deposition following sexual exposure, or laboratory error. Of all 560 type-specific infections among all participants, 86 HR and 73 LR (159 total or 28.4%) infections were found in only one sample ("single positive"). The breakdown of HPV types detected at the sample level analysis excluding single positives (data not shown) was similar to the overall results shown in Table 2. At the sample level excluding single positives, the most frequently detected HR types were HPV-16 (13.7%) and HPV-52 (11.0%). The most frequently detected LR types were HPV-62 (11.4%) and HPV-83 (8.0%).

Analysis at the participant level excluding single positive specimens (data not shown) was also similar to the overall results among participants (Table 4). Percentages varied because of the small denominator (i.e., number of participants). Among participants, excluding single positives, the most frequently detected HR types were HPV-16 (52.5%), HPV-51 (50.0%), and HPV-52, -59, and -66 (47.5% each). The most frequently detected LR types, excluding single positives, were HPV-62 (47.5%), HPV-84 (45.0%), and HPV-83 (40.0%).

As expected, exclusion of single positive samples decreased the average number of HPV types detected per participant. Each participant was positive for an average of 10.0 type-specific HPV infections (range: 2–18; SD: 4.22). Each participant had a mean

Table 5
Vaccine type HPV infections (6, 11, 16, or 18) in participants (N = 40) over the study duration

Number of vaccine type infections	Number of participants	% of participants
0	3	7.5
1	7	17.5
2	17	42.5
3	12	30.0
4	1	2.5
1, 2, 3, or 4	37	92.5

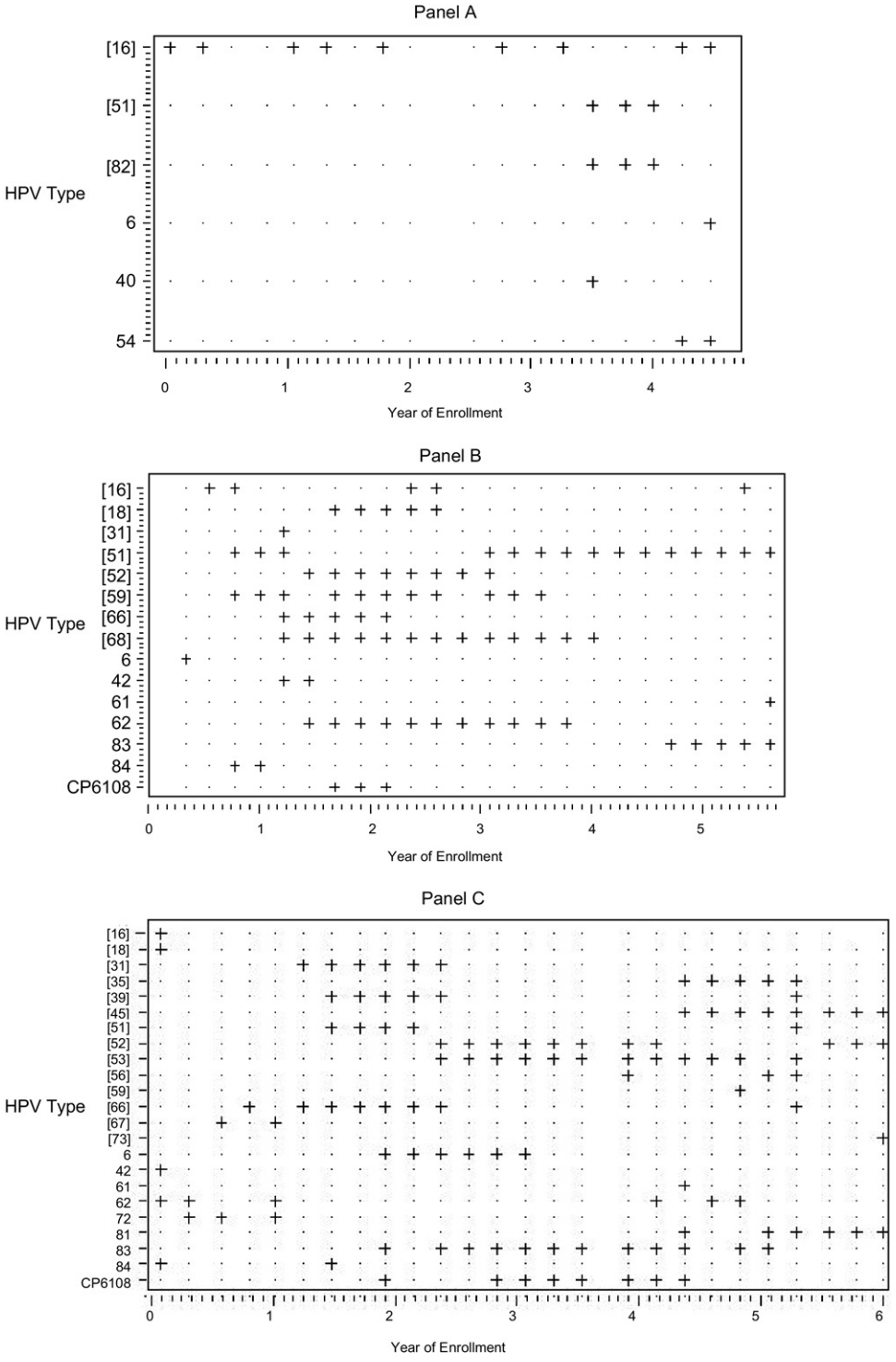

Figure 1. Individual participants in Panels A, B, and C: Plots illustrating examples of sample testing results over the duration of the entire study. X-axis—HPV types (high risk types shown in brackets); Y-axis—Year of enrollment, each vertical line between year marks represents one month; Period (.)—sample negative for the indicated HPV type; Cross (+)—sample positive for the indicated HPV type.

6.1 HR types (range: 1–11; SD: 2.59) and a mean 4.0 LR types (range: 0–9; SD: 2.22). The cumulative prevalence varied for LR types, but not for HR types when single positives were excluded (Table 3).

Duration of HPV infection

The mean duration of the 401 HPV type-specific infections (excluding single positives) was 603.6 days (median: 423 days).

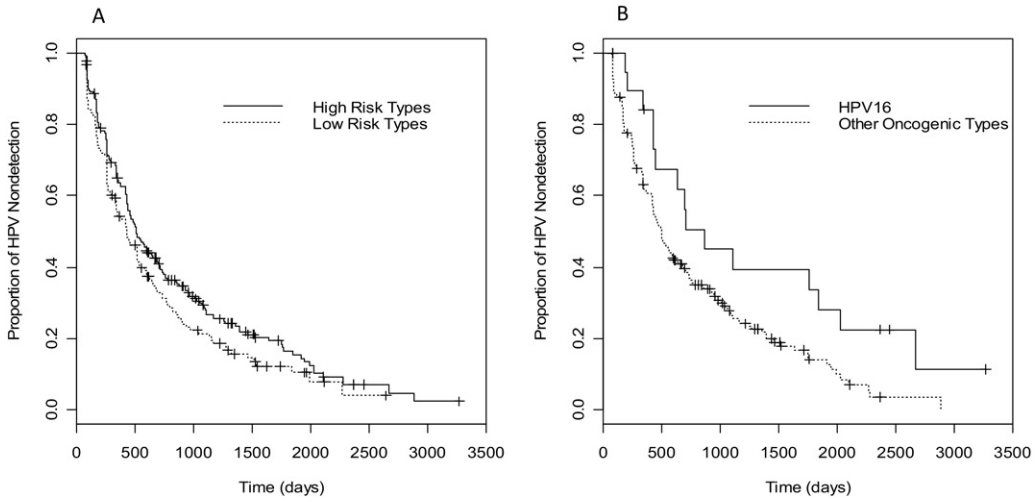

Figure 2. Kaplan–Meier curves illustrating duration of HPV detection. *X* axis: Time in days, *Y* axis: Proportion of infection that become nondetectable. A, Time to nondetection of all high-risk HPV types versus all low-risk HPV types. B, Time to non-detection of HPV-16 versus all other high-risk HPV types.

Among all infections, 242 were HR HPV types, with a mean duration of 655.9 days (median: 433 days), and 159 were LR HPV infections, with a mean length of 524.1 days (median: 334 days). According to the frailty analysis model, HR types had a significantly longer duration of detection ($p = .04$). The hazard ratio of clearance associated with HR HPV was .78; in other words, the instantaneous "hazard" of viral clearance of an HR HPV type was 78% of that of an LR type. HPV-16 had a longer duration of detection (mean: 1,093.4 days; median: 697.0 days) compared with all other HR types (mean: 614.3 days; median: 431.0 days; $p = .01$) (Figure 2).

Discussion

This longitudinal investigation contributes to our knowledge of the natural history of genital tract HPV infections in female adolescents. Although the limited number of subjects potentially reduces the ability to generalize these findings to all adolescent females, each participant contributed several samples over multiple years. This intense characterization of the cohort makes these observations unique among HPV studies. Prior cross-sectional studies provide a limited view of the natural history of HPV infection. For example, in the current study, most participants were eventually infected with at least one vaccine type. This is in contrast to findings of a national cross-sectional study in which only 3.4% of study participants had detectable vaccine type HPV (6, 11, 16, or 18), and .1% had both HPV-16 and -18 [29]. HPV infection was ubiquitous in our cohort, and most participants' cumulative HPV infection history was characterized by multiple HPV HR and LR types. This suggests that many new infections will likely occur even in HPV-immunized women.

Other previous cross-sectional and longitudinal studies illustrate the high prevalence of HPV infection in young women. For example, Moscicki et al found a prevalence of 54.5% for all HPV types and 29.1% for HR HPV types in a cross-sectional study of female adolescents [30]. Tarkowski et al reported a 64% prevalence of HPV in a cross-sectional study of urban adolescent females [2]. In contrast, our previous longitudinal study of young women who were followed up for 2.2 years found a point prevalence of 25%–40% and a cumulative prevalence of 81.7% [13]. In the current longitudinal study, we found an even higher prevalence of HPV in sexually active female adolescents. The point prevalence of infection with any HPV was 55% at enrollment and 78% at the last study visit. The cumulative prevalence of 100% for HPV infection is the highest reported to date for any study. Because infection at a young age may be associated with a higher likelihood of cervical cancer later in life, our findings demonstrate the need for early HPV vaccination before the onset of sexual activity and justify the recommended target range of 11–12-year-old female adolescents [31].

The high prevalence of HPV in our cohort may be due to several factors. First, prolonged follow-up with multiple, frequent sampling made it possible to detect infections that may have been missed in a cross-sectional study or in a longitudinal study with brief follow-up. Second, cells in vaginal swabs may be from the vagina, cervix, or both, thereby potentially increasing the likelihood of obtaining HPV DNA [13]. Third, our cohort self-reported a high rate of prior STIs, suggesting prior high-risk behaviors for acquisition of any STI. Fourth, it is possible that the newer generation LA-HPV assay used may be more sensitive than the older generation assay. Finally, the newer assay detects 10 HPV types not included in the older assay.

We found a broad range of HPV types in our adolescent cohort. The most frequently detected HR types were HPV-16, -59, and -51, each found in >60% of the participants at some point. The most frequently detected LR types were HPV-62 and -6. HPV-62 was not included in the genotyping assay used in our prior study, so it is impossible to compare results. However, in another study, HPV-62 was the most frequently detected LR type [29].

Multiple HPV types were detected in every participant. Some, but not all studies, have concluded that infection with multiple HPV types correlates with the development or progression of cervical dysplasia [11,32–35]. It has also been proposed that infection with multiple types prolongs duration of infection [8,36]. Further investigation of the significance of multiple infections in female adolescents will add to our understanding of HPV epidemiology. Also, studies using the entire cohort of 120 female adolescents will examine whether certain HPV types tend to occur together, or "cluster," and whether the acquisition of cer-

tain HPV types predisposes or protects individuals from subsequent infections.

Infections with HR types were of longer duration as compared with LR types, and duration of both HR and LR infections were longer than those we reported in our prior longitudinal study (median: 188 days for HR and 89 days for LR) [13]. This finding is perhaps in part due to the longer observational window or the enhanced sensitivity of the new LA assay.

Some infections were detected for a specific time, then became undetectable only to become detected again in subsequent samples. This pattern of HPV detection raises the question of whether these infections actually cleared. Further studies in our laboratory will examine such infections to determine whether they truly clear or remain persistent at low levels.

In some subjects, only a single sample was positive for a specific HPV type, which may or may not represent true infection. In our secondary analysis, the exclusion of infections represented by single positive samples did not markedly change our results, suggesting that most of these single positive samples probably represented true infection.

An interesting question relates to HPV detection in sexually naive individuals. We found that two participants had HPV detected in samples collected before their self-reported sexual debut. Explanations for this finding include inaccurate reporting of sexual debut, transmission by contact other than vaginal intercourse, or other transmission during childhood, including nonsexual transmission from parents [37–39]. Correlation with other sexual behaviors may help elucidate a source of the incident HPV infection in nonsexually active participants, and we are pursuing such an analysis in a subset of our cohort.

Cervical cytology results were not included in this analysis. Screening was not a part of the original protocol, but was performed at the discretion of individual clinicians. In addition, there were several changes in recommended screening guidelines during the course of the study, leading to inconsistencies in obtaining cytology in the cohort. A future analysis of cytology data for the entire cohort (N = 120) using medical records is planned.

In conclusion, we found that all participants in our longitudinally followed cohort of female adolescents were infected with multiple HPV types. Our findings support the need to combine nonvaccine strategies with vaccination of young women against HPV before they become sexually active, rather than waiting until an age at which they may become exposed to multiple HPV types. Correlation of these data on HPV infections with behavioral risk factors and cervical cytology will contribute to the evolving comprehension of HPV epidemiology.

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References

[1] Weinstock H, Berman S, Cates W Jr. Sexually transmitted diseases among American youth: Incidence and prevalence estimates, 2000. Perspect Sex Reprod Health 2004;36:6–10.
[2] Tarkowski TA, Koumans EH, Sawyer M, et al. Epidemiology of human papillomavirus infection and abnormal cytologic test results in an urban adolescent population. J Infect Dis 2004;189:46–50.
[3] Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218–26.
[4] Group FIS. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions [Reprint: Ugeskr Laeger 2007;169:3971–4]. N Engl J Med 2007;356:1915–27.
[5] Crosbie EJ, Kitchener HC. Cervarix—A bivalent L1 virus-like particle vaccine for prevention of human papillomavirus type 16- and 18-associated cervical cancer. Expert Opin Biol Ther 2007;7:391–6.
[6] Centers for Disease Control and Prevention. National, state, and local area vaccination coverage among adolescents aged 13–17 years—United States, 2008. Morb Mortal Wkly Rep 2009;58:997–1001.
[7] Franco EL, Villa LL, Sobrinho JP, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999;180:1415–23.
[8] Ho GY, Bierman R, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423–8.
[9] Rousseau MC, Villa LL, Costa MC, et al. Occurrence of cervical infection with multiple human papillomavirus types is associated with age and cytologic abnormalities. Sex Transm Dis 2003;30:581–7.
[10] Mendez F, Munoz N, Posso H, et al. Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. J Infect Dis 2005;192:1158–65.
[11] Bello BD, Spinillo A, Alberizzi P, et al. Cervical infections by multiple human papillomavirus (HPV) genotypes: Prevalence and impact on the risk of precancerous epithelial lesions. J Med Virol 2009;81:703–12.
[12] Sandri MT, Riggio D, Salvatici M, et al. Typing of human papillomavirus in women with cervical lesions: Prevalence and distribution of different genotypes. J Med Virol 2009;81:271–7.
[13] Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis 2005;191:182–92.
[14] Batteiger BE, Tu W, Ofner S, et al. Repeated *Chlamydia trachomatis* genital infections in adolescent women. J Infect Dis 2010;201:42–51.
[15] Gravitt PE, Lacey JV Jr, Brinton LA, et al. Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol Biomarkers Prev 2001;10:95–100.
[16] Lorenzato F, Singer A, Ho L, et al. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. Am J Obstet Gynecol 2002;186:962–8.
[17] Wright TC, Denny L, Kuhn L, et al. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000;283:81–6.
[18] Anhang R, Nelson JA, Telerant R, et al. Acceptability of self-collection of specimens for HPV DNA testing in an urban population. J Womens Health 2005;2002;14:721–8.
[19] Shah K, Daniel R, Tennant M, et al. Diagnosis of human papillomavirus infection by dry vaginal swabs in military women. Sex Transm Infect 2001; 77:260–4.
[20] Safaeian M, Kiddugavu M, Gravitt PE, et al. Comparability of self-collected vaginal swabs and physician-collected cervical swabs for detection of human papillomavirus infections in Rakai, Uganda. Sex Transm Dis 2006;34: 429–36.
[21] Palmisano ME, Gaffga AM, Daigle J, et al. Detection of human papillomavirus DNA in self-administered vaginal swabs as compared to cervical swabs. Int J STD AIDS 2003;14:560–7.
[22] Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357–61.
[23] Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998;36: 3020–7.
[24] Fife KH, Wu JW, Squires KE, et al. Prevalence and persistence of cervical human papillomavirus infection in HIV-positive women initiating highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2009;51: 274–82.
[25] Brown CR, Leon ML, Munoz K, et al. Human papillomavirus infection and its association with cervical dysplasia in Ecuadorian women attending a private cancer screening clinic. Braz J Med Biol Res 2009;42:629–36.

[26] Shew ML, Fortenberry JD, Tu W, et al. Association of condom use, sexual behaviors, and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women. Arch Pediatr Adolesc Med 2006;160:151–6.
[27] Therneau Tag P. Modeling survival data: Extending the cox model: Statistics for biology and health. New York, NY: Springer-Verlag, 2001.
[28] SAS Institute. Base SAS 9.1.3 procedures guide. Cary, NC: SAS Institute Inc, 2006.
[29] Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. JAMA 2007;297:813–9.
[30] Moscicki AB, Ellenberg JH, Vermund SH, et al. Prevalence of and risks for cervical human papillomavirus infection and squamous intraepithelial lesions in adolescent girls: Impact of infection with human immunodeficiency virus. Arch Pediatr Adolesc Med 2000;154:127–34.
[31] Markowitz LE, Dunne EF, Saraiya M, et al. Quadrivalent human papillomavirus vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2007;56:1–24.
[32] Fife KH, Cramer HM, Schroeder JM, Brown DR. Detection of multiple human papillomavirus types in the lower genital tract correlates with cervical dysplasia. J Med Virol 2001;64:550–9.
[33] Wheeler CM, Hunt WC, Schiffman M, Castle PE. Human papillomavirus genotypes and the cumulative 2-year risk of cervical precancer. J Infect Dis 2006;194:1291–9.
[34] Trottier H, Mahmud S, Costa MC, et al. Human papillomavirus infections with multiple types and risk of cervical neoplasia. Cancer Epidemiol Biomarkers Prev 2006;15:1274–80.
[35] Herrero R, Castle PE, Schiffman M, et al. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. J Infect Dis 2005;191:1796–807.
[36] Trottier H, Mahmud S, Prado JC, et al. Type-specific duration of human papillomavirus infection: Implications for human papillomavirus screening and vaccination. J Infect Dis 2008;197:1436–47.
[37] Rintala MA, Grenman SE, Puranen MH, et al. Transmission of high-risk human papillomavirus (HPV) between parents and infant: A prospective study of HPV in families in Finland. J Clin Microbiol 2005;43:376–81.
[38] Pakarian F, Kaye J, Cason J, et al. Cancer associated human papillomaviruses: Perinatal transmission and persistence. Br J Obstet Gynaecol 1994;101:514–7.
[39] Doerfler D, Bernhaus A, Kottmel A, et al. Human papillomavirus infection prior to coitarche. Am J Obstet Gynecol 2009;200:e481–5.