MAJOR ARTICLE

A Prospective Study of Age Trends in Cervical Human Papillomavirus Acquisition and Persistence in Guanacaste, Costa Rica

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(See the editorial commentary by Winer and Koutsky, the article by Herrero et al., and the brief report by Dunne et al., on pages 1787–9, 1796–807, and 1817–9, respectively.)

Background. Cross-sectional human papillomavirus (HPV) DNA prevalence peaks at young ages, reflecting sexual acquisition and typically rapid clearance. In some populations, HPV prevalence demonstrates a second peak in older women. Longitudinal data may help to explain this second peak.

Methods. We followed a population-based cohort of 7237 women in Guanacaste, Costa Rica, in which we had previously observed a second peak in the baseline HPV prevalence in older women. We tested for >40 HPV types by polymerase chain reaction. We analyzed age-specific patterns of acquisition and persistence 5–7 years after enrollment for individual HPV types.

Results. At enrollment and follow-up, cross-sectional data revealed U-shaped age-specific HPV prevalence curves for virtually every type, with higher prevalences in the younger and older women than in the middle-aged women. Prospectively, acquisition of types decreased significantly as women aged ($P_{\text{Trend}} < .05$, for both), with the highest peak in young women and a secondary minor peak in older women. Type-specific persistence of HPV increased with age ($P_{\text{Trend}} < .0001$). Overall, HPV acquisition predominated at younger ages, whereas persistent infections gradually became more prominent with age ($P_{\text{Trend}} < .0001$).

Conclusions. Newly apparent infections decreased, whereas persistence increased, with age; this latter tendency supports the utility of HPV screening in older women.

The earliest studies of oncogenic human papillomavirus (HPV) DNA prevalence reported a steady decrease with age, a pattern concordant with viral clearance and reduced exposure to new HPV types. This pattern raised the possibility of infrequent, accurate, and cost-effective HPV screening in older women, to identify and prevent cervical cancer [1, 2].

However, some studies in regions where the risk of HPV infection is high—such as our study in Guanacaste, Costa Rica—have observed a U-shaped age-specific HPV prevalence curve [3–7] or even no change [8], rather than a steady decrease with age. Viral prevalence is the product of incidence (acquisition of new infections) and duration (persistence). Possible mechanisms that would increase acquisition include age-related female or male sexual behaviors, increased detection of HPV infection due to age- or menopause-related changes of the cervicovaginal epithelium, and age-related immune senescence leading to increased reactivation of latent infections (and so leading to increased detection of seemingly new infections). Increased viral persistence in older women could result from a decreased ability of older women to clear recently ac-

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quired HPV infections or from the gradual predominance (i.e., greater proportion) of long-duration infections from earlier exposures over more recent and more transient infections. Higher HPV prevalences could also result from a cohort effect, such that the women of older generations experience more viral exposure or greater persistence than do the women of younger generations.

Determination of the components of the observed age-specific HPV prevalence patterns is important for identification of optimal prevention strategies. In particular, if new infections appear frequently as women age, the practical effectiveness of screening infrequently for oncogenic HPV types as a mainstay of cervical cancer prevention might be less than anticipated. However, comprehension of age-specific prevalence curves requires prospective data from population-based cohorts, which are only now reaching sufficient follow-up time.

To help clarify the origins of the second, pronounced peak of HPV infection in older women in Guanacaste—which was confirmed by our comprehensive analysis presented in the accompanying article in this issue of *The Journal of Infectious Diseases* [4]—we tested specimens from cohort members 5–7 years after enrollment. This enabled us to further explore the U-shaped age-specific HPV prevalence curve and to separate the independent effects that type-specific acquisition and persistence/clearance have on age-specific prevalence patterns.

PARTICIPANTS, MATERIALS, AND METHODS

Study population. The Proyecto Epidemiológico Guanacaste is a population-based study in a province of Costa Rica where rates of cervical cancer are historically high [3, 4, 9, 10]. The recruitment of a representative random population sample, multimethod screening, and follow-up have been detailed elsewhere [4, 9, 10]. We obtained consent from all participants, in accordance with the guidelines of the US Department of Health and Human Services. Institutional review boards at the U.S. National Cancer Institute and in Costa Rica approved this study.

The full study population included 10,077 women (10,049 when 28 supplemental participants with cancer are excluded). We prospectively followed 8545 of them, after excluding women who had undergone a hysterectomy ($n = 630$), who were virgins ($n = 583$), and who had refused a pelvic examination ($n = 291$) [4, 9, 10]. For the present analysis, we further excluded women who were missing either enrollment ($n = 32$) or follow-up ($n = 1113$) polymerase chain reaction (PCR) results. Of those women missing follow-up PCR results, 801 were lost to follow-up, a group that was predominantly comprised of the youngest women (who tended to seek work outside the province) and the oldest women ($P < .0001$, Pearson χ^2 test for 7 age strata). We also excluded women who were diagnosed with and treated for cervical intraepithelial neoplasia (CIN) grade 2 or higher at enrollment ($n = 139$) [3, 11]. Finally, because we were studying HPV acquisition and clearance, we excluded women with clearly missed prevalent CIN3 and cancer diagnosed during follow-up, on the basis that it implies both viral prevalence and viral persistence ($n = 24$) [12]. The remaining analytic group contained 7237 women (see figure 1 in the accompanying article in this issue of the *Journal* for a diagram of the study population [4]).

We used follow-up specimens obtained during return visits 5–7 years after enrollment, except for specimens from 233 women (3.2%) who had been censored earlier during follow-up; for these women, we tested the last available specimen. The reasons for early censoring included incident CIN3 ($n = 15$), suspect CIN2 lesions that were later ruled out on pathological review ($n = 131$), and death, hysterectomy, serious illness, and refusal to continue participation ($n = 87$) The mean interval between collection of enrollment and follow-up specimens was 5.6 years (SD, 1.2 years), with a median interval of 5.1 years (range, 0.5–8.2 years).

HPV DNA detection. HPV DNA was detected in exfoliative cervical specimens stored in specimen-transport medium (STM; Digene) by use of MY09/M11 L1 consensus primer PCR with AmpliTaq Gold polymerase, as described elsewhere [4, 13, 14]. HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and AE2 (82 subtype) were considered to be oncogenic [15].

Enrollment specimens were tested in 6 large batches, and the follow-up specimens were tested in 6 other batches. To assess batch-to-batch variability that could influence the prospective analyses, we placed 128 masked aliquots of enrollment specimens into the follow-up batches. Furthermore, we retested all specimens with residual volume that had initially tested positive for HPV-16 ($n = 255$) in an additional batch that included both enrollment and follow-up specimens from the same woman, to exclude the possibility of confounding of HPV-16 data by batch effects.

The batch-to-batch variability data suggested that the follow-up testing might have been less sensitive than the enrollment testing ($P = .025$, for oncogenic types; $P = .13$, for nononcogenic types; McNemar χ^2 test for HPV positivity). However, there was no association between age and batch number. Thus, batch effects could not explain age trends in prevalence or the relative contributions of persistence and acquisition to prevalence.

Statistical methods. For the present analysis, we calculated the percentages of acquisition, clearance, and persistence for each HPV type individually. HPV persistence was defined as a positive result for the same type at both enrollment and follow-up (+/+). HPV clearance and acquisition were defined as a positive result for a specific type at enrollment only (+/−) and at follow-up only (−/+), respectively. Analyses of the natural history of HPV infection are complicated by the relative rarity

of each individual type and the common occurrence of multiple-type infections. In the present analysis, 31.8% ($n = 491$) of infections at enrollment and 30.2% ($n = 347$) of infections at follow-up occurred concurrently with at least 1 other type. More specifically, 21.6% ($n = 185$) and 17.4% ($n = 115$) of infections with oncogenic types occurred concurrently with other oncogenic types at enrollment and follow-up, respectively. For infections with nononcogenic types, 19.8% ($n = 193$) of enrollment and 19.1% ($n = 128$) of follow-up infections occurred concurrently with other nononcogenic types.

Rather than compute statistics such as the percentage of women infected with ⩾1 oncogenic type, we chose to analyze each HPV type individually. This approach is justified by data suggesting that HPV infections act independently [16, 17]. In the present analysis, a woman with a multiple-type infection contributed to the type-specific tables in the manner illustrated by the following hypothetical example of a woman infected with HPV-16 and HPV-61 at enrollment and with HPV-16 and HPV-18 at follow-up: her data would contribute to the +/+ cell for the HPV-16 cross-tabulation, to the +/− cell for the HPV-61 cross-tabulation, to the −/+ cell for the HPV-18 cross-tabulation, and to the −/− cell for the cross-tabulation of all other types. Considering each infection as an independent event [16] permitted us to compute the total numbers and percentages of acquisition, persistence, and clearance for >40 such tables, 1 for each type.

We observed that HPV types within each of the 2 broad categories of etiologic and clinical relevance—that is, the oncogenic and nononcogenic types—shared the same patterns. Thus, for clarity, we present the average statistics for the types combined in these 2 groups, rather than details for each individual type. For example, we present the mean of the persistences for all types in the oncogenic group; to compute this mean, we summed the type-specific +/+ counts for all 17 types considered to be oncogenic and divided the resultant value by the total number of oncogenic types detected at enrollment (+/+ plus +/−). To supplement the presentation of the mean data of the grouped types, we also present individual data for the most prevalent HPV type in the oncogenic and nononcogenic categories—HPV-16 and HPV-61, respectively.

The women's ages at enrollment and follow-up were used to generate cross-sectional age-specific HPV prevalence curves. Except where noted, age groups were defined on the basis of age at enrollment: <25 years ($n = 893$ [12.3%]), 25–34 years ($n = 2259$ [31.2%]), 35–44 years ($n = 1821$ [25.2%]), 45–54 years ($n = 1051$ [14.5%]), 55–64 years ($n = 677$ [9.4%]), and ⩾65 years ($n = 536$ [7.4%]). Use of 5-year rather than 10-year intervals for age decreased the stability of the estimates and did not change the observations. Therefore, we present the smoother curves that resulted from use of the 10-year intervals.

McNemar's χ^2 test was used to compare the detection of new HPV infections and cleared infections (absolute numbers) within each age group. The Mantel extension of the χ^2 test was used to evaluate age trends for acquisition, persistence, and the relative contributions of persistent and acquired infection to prevalence at follow-up. $P < .05$ (2-sided) was considered to be statistically significant.

RESULTS

Cross-sectional analyses of age-specific HPV prevalence. As described elsewhere, we previously observed a U-shaped age-specific HPV prevalence curve in the enrollment data [3, 4] (figure 1). The mean prevalences of oncogenic and nononcogenic HPV types were high in the youngest women but decreased in the middle-aged women, with a second peak in the older women. The second peak was more pronounced for nononcogenic HPV types than for oncogenic types. In the follow-up data, the increase was observed again, although it was somewhat less pronounced. We observed similar patterns for the 2 representative types, HPV-16 and HPV-61.

Prospective analysis of net change in HPV positivity by age group. The prospective analysis first assessed the net change in HPV positivity by age group, with each woman's status at enrollment compared with that at follow-up (table 1). For the oncogenic types, the number cleared was larger than the number acquired in each age group, with generally significant results. For HPV-16, clearance and acquisition were similar for each age group, except for the ⩾65-year-old women, for whom clearance was significantly more common than acquisition ($P = .02$). For the nononcogenic types, clearance was again more common than acquisition in each age group and was statistically significant for virtually all age groups.

Prospective analyses of HPV acquisition and persistence by age group. HPV acquisition generally decreased with increasing age for the oncogenic types, the nononcogenic types, HPV-16, and HPV-61 (figure 2). Of note, except for HPV-61, slight increases in acquisition were observed in the middle-aged women, which were followed by further decreases in the older women; for HPV-61, acquisition did not decrease in the older women. In contrast, HPV persistence increased significantly with age for the oncogenic types, the nononcogenic types, HPV-16, and HPV-61 ($P < .0001$; figure 3).

To summarize the 2 trends that might contribute to the U-shaped age-specific HPV prevalence curve seen in the follow-up data, the younger women were more likely to acquire HPV infection during follow-up than were the older women, who were more likely to remain persistently positive for HPV types they were infected with at enrollment. As a summary statistic, we calculated the percentage of infections apparent at follow-up that resulted from persistence (figure 4). The trend toward greater viral persistence (vs. acquisition) in the older women was highly significant for both the oncogenic types ($P_{\text{Trend}} <$

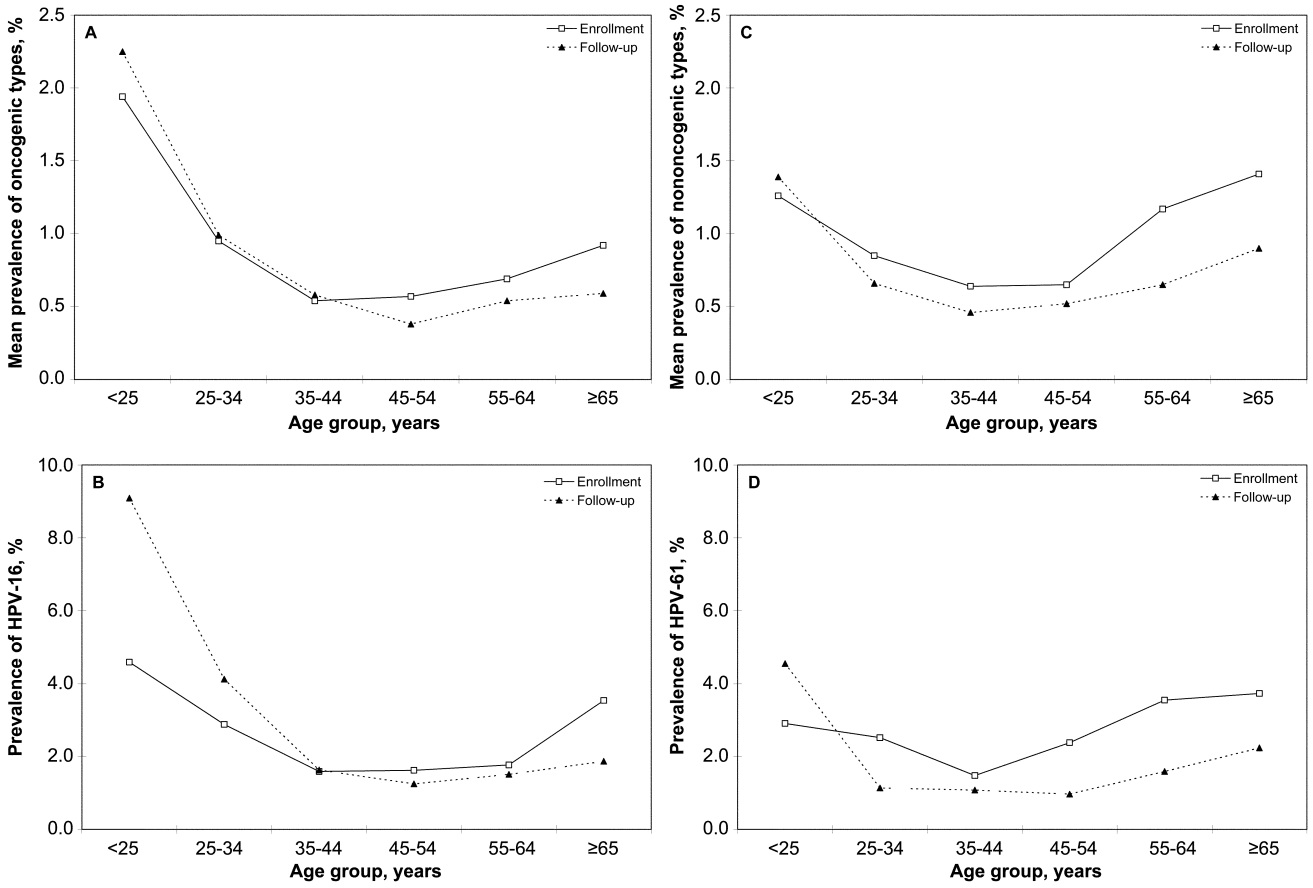

Figure 1. Prevalence of human papillomavirus (HPV) by age group at both enrollment and follow-up. *A,* Mean prevalence of oncogenic types. *B,* Prevalence of HPV-16. *C,* Mean prevalence of nononcogenic types. *D,* Prevalence of HPV-61. Age groups are defined on the basis of age at the time the specimen was collected. Note that the scales are not uniform among the 4 panels.

.0001) and the nononcogenic types ($P_{\text{Trend}} < .0001$). Slightly more than 50% of infections were persistent in the women ⩾65 years old. This trend was especially strong for the most prevalent oncogenic type, HPV-16: the percentages of infections at follow-up that were persistent were 15.2% for the <25-year-old women, 25.4% for the 25–34-year-old women, 26.9% for the 35–44-year-old women, 41.7% for the 45–64-year-old women (groups combined), and 70% for the ⩾65-year-old women ($P_{\text{Trend}} < .0001$; age groups are based on enrollment).

To confirm our observations, we also examined longitudinal HPV-16 PCR results performed in a single batch, thereby avoiding any batch-to-batch variability that might have contributed to the observed patterns (data not shown). We observed HPV-16 patterns for the longitudinal PCR results that were similar to those observed in the enrollment and follow-up data, demonstrating the robustness of these results despite a slightly lower analytic sensitivity in the testing of follow-up specimens. We had interval data on 44 actively followed (with visits every 6–12 months) women who were HPV-16 positive at both enrollment and follow-up. Only 5 (11%) of these women lost and then regained an HPV-16 infection during the interval, and 3 of these 5 had only a single HPV-16 negative result within a series of positive results.

DISCUSSION

By retesting specimens obtained during follow-up visits from >7000 women in a random sample of a single, stable community, we have been able to show that 5-year persistence of HPV infection increases and that acquisition of HPV infection decreases with age. Our data suggest that the new appearance of HPV types that were not present 5 years earlier is unlikely to explain the second peak in HPV prevalence in older women [4]. Any significant acquisition- or reacquisition-related phenomena (e.g., new exposures to HPV via new male sex partners or their partners becoming infected via new partners, an increased ability to detect HPV in the cervical epithelium of aging postmenopausal women, and age-related immune senescence leading to increased reactivation of latent infections) would result in clear and continuing increases in newly apparent HPV infections in older HPV negative women who entered the prospective follow-up. Instead, follow-up testing revealed that HPV

Table 1. Comparisons of human papillomavirus (HPV) DNA status (positive [+] or negative [−]) for any oncogenic type, HPV-16 (the most prevalent oncogenic type), any nononcogenic type, and HPV-61 (the most prevalent nononcogenic type) at enrollment and follow-up.

Category or type, age group	−/−	+/−	−/+	+/+	P^a
Oncogenic types					
<25 years	14,743 (97.1)	272 (1.8)	144 (0.9)	22 (0.1)	**<0.0001**
25–34 years	37,791 (98.4)	318 (0.8)	249 (0.6)	45 (0.1)	**0.004**
35–44 years	30,660 (99.0)	145 (0.5)	129 (0.4)	23 (0.1)	0.3
45–54 years	17,708 (99.1)	86 (0.5)	57 (0.3)	16 (0.1)	**0.02**
55–64 years	11,379 (98.9)	58 (0.5)	51 (0.4)	21 (0.2)	0.5
⩾65 years	9002 (98.8)	56 (0.6)	26 (0.3)	28 (0.3)	**0.0009**
HPV-16					
<25 years	813 (91.0)	34 (3.8)	39 (4.4)	7 (0.8)	0.6
25–34 years	2152 (95.3)	50 (2.2)	42 (1.9)	15 (0.7)	0.4
35–44 years	1774 (97.4)	22 (1.2)	18 (1.0)	7 (0.4)	0.5
45–54 years	1028 (97.8)	12 (1.1)	6 (0.6)	5 (0.5)	0.2
55–64 years	656 (96.9)	6 (0.9)	9 (1.3)	6 (0.9)	0.4
⩾65 years	514 (95.9)	12 (2.2)	3 (0.6)	7 (1.3)	**0.02**
Nononcogenic types					
<25 years	16,635 (98.1)	201 (1.2)	116 (0.7)	13 (0.1)	**<0.0001**
25–34 years	42,335 (98.7)	340 (0.8)	212 (0.5)	23 (0.1)	**<0.0001**
35–44 years	34,221 (98.9)	204 (0.6)	145 (0.4)	17 (0.0)	**0.001**
45–54 years	19,749 (98.9)	109 (0.5)	86 (0.4)	20 (0.1)	0.1
55–64 years	12,639 (98.3)	124 (1.0)	71 (0.6)	27 (0.2)	**0.0001**
⩾65 years	9991 (98.1)	99 (1.0)	47 (0.5)	45 (0.4)	**0.0009**
HPV-61					
<25 years	850 (95.2)	26 (2.9)	17 (1.9)	0 (0.0)	0.2
25–34 years	2179 (96.5)	55 (2.4)	23 (1.0)	2 (0.1)	**0.0003**
35–44 years	1783 (97.9)	22 (1.2)	11 (0.6)	5 (0.3)	0.06
45–54 years	1019 (97.0)	21 (2.0)	7 (0.7)	4 (0.4)	**0.008**
55–64 years	647 (95.6)	17 (2.5)	6 (0.9)	7 (1.0)	**0.02**
⩾65 years	511 (95.3)	13 (2.4)	5 (0.9)	7 (1.3)	0.06

NOTE. Data are no. (%) of infections, unless otherwise noted. For single types HPV-16 and HPV-61, the no. of infections equals the no. of participants. For HPV groups of oncogenic and nononcogenic types, the no. of infections equals the no. of types in each group (e.g., 17 oncogenic types) multiplied by the number of patients (e.g., in women <25 years old, 17 oncogenic types × 893 patients = 15,181 infections). HPV persistence was defined as a positive result for the same type at both enrollment and follow-up (+/+). HPV clearance and acquisition were defined as a positive result for a specific type at enrollment only (+/−) and at follow-up only (−/+), respectively. Age groups are defined on the basis of age at enrollment.

[a] For comparisons between clearance (+/−) and acquisition (−/+), by the McNemar χ^2 test. Boldface indicates that +/− and −/+ counts were significantly different ($P < .05$).

infections tended to clear (+/−) more often than new (or recurrent) HPV infections appeared (−/+), except in the young women in whom first acquisitions tend to occur. The nonsignificant slight increase in newly apparent infections that we noted in the middle-aged women was too small and inconsistent in the older women to contribute much to the U-shaped HPV prevalence curve. In short, because prevalence equals incidence multiplied by duration (persistence) for each age group, our data support a stronger role for viral persistence—a necessary step in the development of cervical cancer [18]—than for acquisition in older women.

Except for a small group of women tested serially for HPV-16, our data were limited to 2 measurements obtained ~5 years apart. We report that, at least for HPV-16, 2 measurements could be interpolated to intervening HPV results. However, 5 years are insufficient to explain definitively the natural history of HPV infection over the decades of women's lives. For example, when we examined the HPV prevalence patterns using finer divisions of age, there was some weak evidence that the inflection point at which the age-specific prevalence in Guanacaste began to increase again was shifted 5–10 years later in the follow-up data, compared with that in the enrollment data.

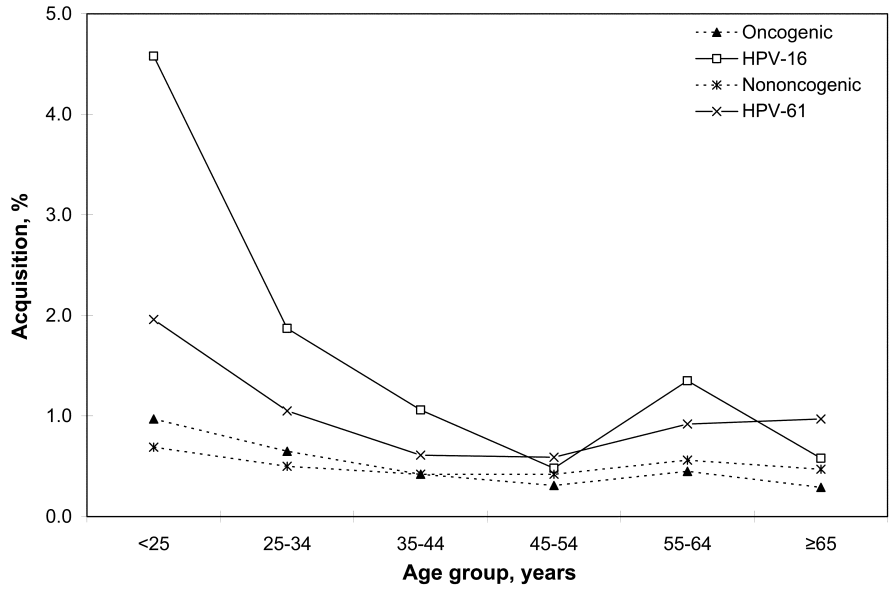

Figure 2. Acquisition of human papillomavirus (HPV) by age group. Age groups are defined on the basis of age at enrollment.

This could suggest a cohort effect, a phenomenon in which the women born at a certain time in a region experience risks that are different from those in the women born at other times. For example, in the United States, the women who grew up after the widespread acceptance of oral contraceptives represent a clear cohort that should be distinguished from cohorts of older women, whose practices with respect to sex and contraceptive use were different. We do not know of any cohort effect that would explain a higher HPV prevalence in older (≥55 years) women versus middle-aged (35–54 years) women in Guanacaste, but we hope to eventually reexamine this possibility in an age-period-cohort analysis using data from 10 years of follow-up. If a cohort effect does explain the elevated prevalence in older women today, it would be expected that the prevalence (especially of nononcogenic types) had been extremely high in this cohort of women when they were young.

We do not know the biological explanation for the striking increase in HPV persistence with age. Stratification by whether women had had new sex partners during follow-up did not appreciably affect these findings, except to slightly decrease the

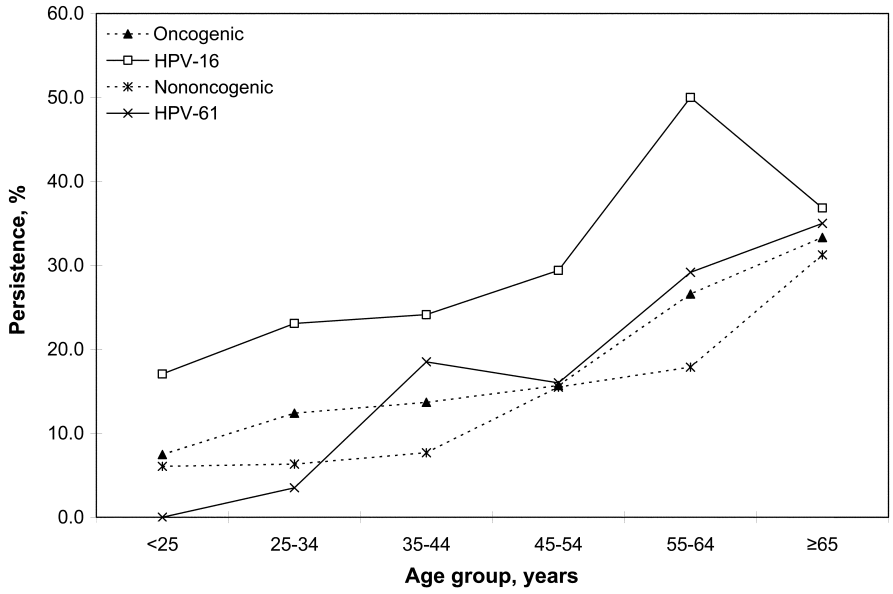

Figure 3. Persistence of human papillomavirus (HPV) by age group. Age groups are defined on the basis of age at enrollment.

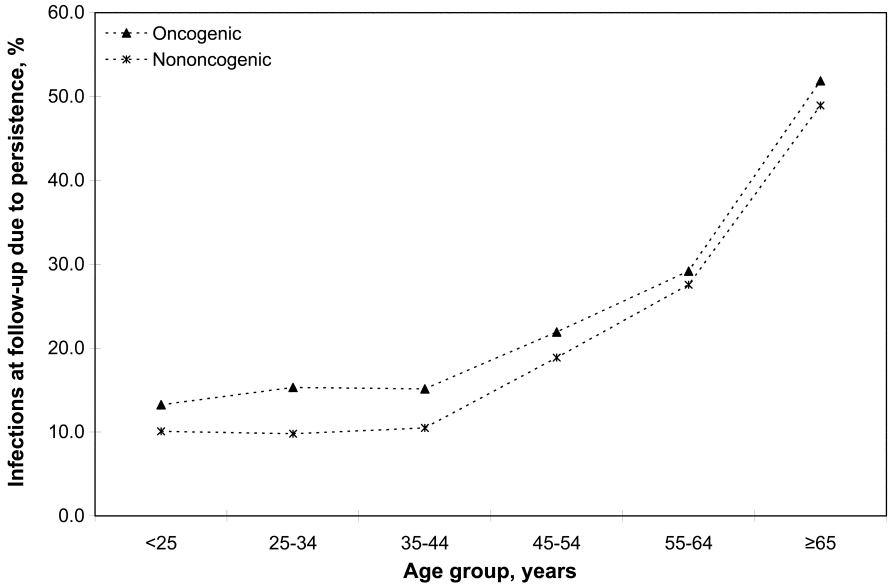

Figure 4. Percentage of human papillomavirus infections at follow-up that were the result of persistence from enrollment, stratified by age group. Age groups are defined on the basis of age at enrollment.

ratio of persistence to acquisition of HPV during follow-up, suggesting that these observed patterns represent true HPV persistence (data not shown). The trend is consistent with age-related immune senescence affecting HPV clearance. Alternatively, prevalent infections detected at baseline in older women may reflect a greater likelihood of long-duration (persistent) infections than prevalent infections detected in the younger women, with a concomitant greater likelihood of persistence. Prospective studies of incident infections that are acquired under observation in which viral variants and host factors are taken into consideration will be needed to explain the relationship between greater persistence and older age [19, 20].

We note that, although U-shaped age-specific HPV prevalence curves were observed in the follow-up data, they were somewhat less pronounced than those observed in the enrollment data. At the initiation of the study, cervical cancer rates were very high in Guanacaste [9], a fact that can largely be attributed to ineffective Pap screening in this region. The reasons for the diminution in the follow-up U-shaped age-specific HPV prevalence curves remain unexplained, however, although it could be the result of more effective screening.

The strength of the present analysis is the use of a random sample, population-based cohort that had >90% participation at enrollment and almost 90% participation during follow-up [10], eliminating biases. Preferential losses in the youngest and oldest age groups were unlikely to influence the observed patterns, because HPV status was not used in the clinical management of these women and therefore was unknown to them.

Interestingly, the U-shaped age-specific HPV prevalence curve was more pronounced for the nononcogenic types than for the oncogenic types, as exemplified in the HPV-61 versus HPV-16 data. We cannot explain the difference, but, on the basis of these data, we suggest that differences might exist between the epidemiologic profiles of the HPV types in the 2 risk categories, even though all types are sexually transmitted. We have recently observed that some nononcogenic types are more commonly found in vaginal specimens from women who have undergone a hysterectomy and have no cervix than in cervical specimens from women who have not undergone a hysterectomy [21]. Thus, it is plausible that some HPV types survive preferentially in vaginal squamous epithelium, compared with epithelium of the cervix or the squamocolumnar junction. Reduced size of the squamocolumnar junction and replacement of the cervical mucosa by atrophic stratified squamous epithelium might explain the second peak in older women, which may be consistent with the second peak in acquisition in middle-aged women. We are now investigating this possibility.

Additional studies will be needed to understand these patterns more fully. We will use Markov chain modeling [10] to better understand the interaction of types in relationship to their natural history and cytologic/histologic morphology. We are also conducting a follow-up study in older women from this cohort to further explore HPV prevalence patterns and the immune correlates of these patterns. Finally, we are examining patterns of persistence and progression to CIN3 and cancer for each type [22].

Our results support the utility of HPV screening in women who have passed the first peak of HPV prevalence and the possibility of substantially lengthened screening intervals for women. Cervical cancer is very uncommon in women <30 years

old; thus, HPV screening of women starting at age 30–35 has been proposed by several organizations [23–25]. The residual concern has been our poor understanding of the importance of newly apparent HPV infections to cervical carcinogenesis in older women. Previous studies have shown that most HPV infections tend to clear spontaneously [19, 20, 26, 27] and that cervical neoplasia results from persistently detectable oncogenic HPV infection [28–30]. If we had observed a second peak of acquisition or viral reemergence underlying the cross-sectional U-shaped HPV prevalence curve, the negative predictive value of a negative screen in middle-aged women would have been less effective.

However, specific recommendations will need to be crafted carefully, given the holes that remain in our knowledge of infections with various HPV types and their associated risks in older women. Although persistence gradually predominated over acquisition in the groups of older women, there were oncogenic infections in older women that appeared at follow-up but not at enrollment 5 years earlier. We are examining the possibility of measurement error by testing all available longitudinal specimens. Nevertheless, it is unlikely that one-time HPV screening of older populations can prevent all subsequent cervical cancer, even with a sensitive HPV test. The degree of negative predictive value that will be provided by different testing protocols and screening intervals remains to be established in future longitudinal studies.

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