Longitudinal Study of Human Papillomavirus Persistence and Cervical Intraepithelial Neoplasia Grade 2/3: Critical Role of Duration of Infection

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Background
The natural history of human papillomavirus (HPV) infections in older women is critical for preventive strategies, including vaccination and screening intervals, but is poorly understood. In a 7-year population-based cohort study in Guanacaste, Costa Rica, we examined whether women’s age and the duration of carcinogenic HPV infections influenced subsequent persistence of infection and risk of cervical intraepithelial neoplasia grade 2 (CIN 2) or worse disease.

Methods
At enrollment, of the 9466 participants eligible for pelvic examination, 9175 were screened for cervical neoplasia using multiple methods; those with CIN 2 or worse disease were censored and treated. Participants at low risk of CIN 2 or worse (n = 6029) were rescreened at 5–7 years (passively followed), whereas higher-risk participants (n = 2115) and subsets of low-risk women (n = 540) and initially sexually inactive women (n = 410) were rescreened annually or semiannually (actively followed) for up to 7 years. HPV testing was done using a polymerase chain reaction–based method. We determined, by four age groups (18–25, 26–33, 34–41, and ≥42 years), the proportion of prevalent infections (found at baseline) and newly detected infections (first found during follow-up) that persisted at successive 1-year time points and calculated absolute risks of CIN 2 and CIN grade 3 (CIN 3) or worse during follow-up. P values are two-sided.

Results
Regardless of the woman’s age, newly detected infections were associated with very low absolute risks of persistence, CIN 2, or worse disease. For newly detected infections, the rate of progression to CIN 2+ (or CIN 3+), after 3 years of follow-up, was not higher for women aged 34 years and older than for younger women. Moreover, rates of newly detected infections declined sharply with age (in the actively followed group, at ages 18–25, 26–33, 34–41, and ≥42 years, rates were 35.9%, 30.6%, 18.1%, and 13.5%, respectively; \( P < .001 \)). Among prevalent infections, persistent infections among older women (≥42 years) was higher than that among younger age groups or new infections at any age (\( P < .01 \) for comparison of eight groups). Most (66 of 85) CIN 2 or worse detected during follow-up was associated with prevalent infections. Only a small subset (25 of 1128) of prevalent infections persisted throughout follow-up without apparent CIN 2 or worse.

Conclusions
The rate of new infections declines with age, and new infections typically do not progress to CIN 2 or worse disease in older women; thus, overall potential benefit of prophylactic vaccination or frequent HPV screening to prevent or detect new carcinogenic HPV infections at older ages is low.


Persistent infection with any of approximately 10–15 carcinogenic genotypes of human papillomavirus (HPV) can cause cervical cancer and its immediate precursor, cervical intraepithelial neoplasia grade 3 (CIN 3). Notably, infection with HPV-16 accounts for half of all cervical cancer cases (1). CIN 3 can develop within a few years of HPV infection; the time elapsed between recognition of infection and CIN 3 diagnosis depends not only on biological determinants (eg, characteristics of the virus, host, or environment) but also on the intensity and sensitivity of clinical methods of HPV detection and CIN 3 diagnosis (2–5). Typically, lesions expand slowly and superficially for many years before becoming invasive; approximately one-third of large and advanced CIN 3 lesions lead to invasive cancer (6). Although HPV-induced precancerous lesions can, in rare instances, rapidly lead to cancer, the average total time from infection with a carcinogenic HPV to invasive cervical cancer, if it occurs, is 25–30 years or more.

Understanding the central role of HPV infection as a cause of cervical carcinogenesis has led to introduction of prophylactic vaccination programs and implementation of cervical screening intervals for women of reproductive age. The natural history of HPV persistence and CIN 3 diagnosis depends not only on biological factors but also on the intensity and sensitivity of clinical methods of HPV detection and CIN 3 diagnosis (2–5). Typically, lesions expand slowly and superficially for many years before becoming invasive; approximately one-third of large and advanced CIN 3 lesions lead to invasive cancer (6). Although HPV-induced precancerous lesions can, in rare instances, rapidly lead to cancer, the average total time from infection with a carcinogenic HPV to invasive cervical cancer, if it occurs, is 25–30 years or more.
HPV vaccines and use of HPV tests for screening and triage. The absence of HPV infection indicates extremely low risk of imminent diagnosis of CIN 3 or cancer, but one-time detection of HPV does not necessarily imply very high risk over the next few years. Transiently detectable infections with carcinogenic HPV types are extremely common, particularly among sexually active adolescents and young women (3,7–9). The very small percentage (<10%) of infections that persist beyond 2–3 years have high subsequent risk of continued persistence, diagnosis of CIN 3, and eventual cervical cancer (2,10,11). Therefore, a focus on HPV persistence, and avoidance of overreaction to HPV infections that are likely to resolve spontaneously, is essential for a rational introduction of HPV testing into cervical cancer screening programs (2).

Although long-term persistence of carcinogenic HPV genotypes is the cause of cervical cancer worldwide, age-specific prevalence curves at older ages vary widely by region (12). This variation by age and geography must be better understood before prophylactic HPV vaccines and screening become mainstays of cervical cancer prevention. In many developed countries, prevalence of HPV infections decreases substantially and steadily with age, as would be expected for a typically transient sexually transmitted infection; however, in a few countries, no decline has been observed, and in others, a second peak at older ages has been described (12,13).

It is important to clarify the relationship between women’s age, the duration of carcinogenic HPV infections, and risk of cervical cancer. We previously described the natural history of HPV infection among the 10 049 women of a population-based cohort from Guanacaste, Costa Rica, using assessment at two time points spaced 5–7 years apart (14,15). In that analysis, infections that were detected as prevalent at enrollment were more likely to persist as women’s age increased, whereas the appearance of previously undetected infections declined steadily with age (16). We postulated that newly appearing infections in older women are a mixture of new infections and previously latent infections that were reactivated because of failure of immune control after a period of undetectability. We considered that the increased rate of persistence of prevalently detected infections with age was possibly because of either weakening immune response or a form of viral selection in which the infections found as prevalent among older women represented those that had evaded immune control for many years and thus were more likely to continue to persist.

Since our last report, completion of the full longitudinal testing of all specimens from women in the Guanacaste cohort has allowed us to compare the outcomes of prevalently detected infections (ie, those detected at baseline) with newly detected infections (ie, those first found during follow-up). Understanding infections found in older women requires longitudinal follow-up studies to differentiate prevalent infections, which are more likely to already be old, from newly detected infections and to assess risk of persistence and progression. Available studies (12,16–20) provided no clear consensus on the impact of age on the natural history of HPV infection, and we were particularly interested in the natural history and predictive value of newly detected infections because HPV vaccines are prophylactic, so determining which age groups to vaccinate is an important question. In this study, we examined in particular whether older women respond differently from young women to what appear to be new HPV exposures, both in terms of subsequent persistence of the infections and in terms of risk of CIN grade 2 or worse (CIN 2+).

Methods

Study Design and Population

Between June 1993 and December 1994, a population-based cohort was assembled in Guanacaste, Costa Rica, to study the natural history of HPV infection and cervical neoplasia; 10 049 women who were 18 years or older were recruited, with a participation rate of 93.6%. Details regarding the cohort design, participation rate of 93.6%. Details regarding the cohort design, methods, and written informed consent have been described elsewhere (14).

At enrollment, women were screened with cervicography, three kinds of cytology (ie, conventional, liquid based, and a computer-assisted method called PapNet), a visual examination by a nurse, and an early HPV test, the hybrid capture test (HCT) test (Digene, Gaithersburg, MD, now Qiagen), which detected 11 carcinogenic types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) at a threshold of 10 pg/mL of DNA from exfoliated cervical cells preserved in specimen transport medium (STM; Digene,
Exfoliated cells for conventional and liquid-based cytology (ThinPrep Cyte Corporation, Marlborough, MA) were collected monthly by the National Cancer Institute and Casa Rican Institutional Review Board. Women who tested negative in the screen and who were sent to confirm the sensitivity of the referral algorithm (among whom, no CIN 2+ was found).

After enrollment (Table 1), 955 women were censored from the active cohort. The study protocol was reviewed and approved annually by the National Cancer Institute and a Costa Rican Institutional Review Board.

### Table 1. Demographic characteristics and prevalent vs newly detected carcinogenic human papillomavirus (HPV) infections among women in the Guanacaste cohort by screening group*

<table>
<thead>
<tr>
<th>Screening group</th>
<th>No. of women</th>
<th>Mean age at enrollment, y</th>
<th>Median annual-time-bins, y</th>
<th>Baseline prevalence‡ (95% CI)</th>
<th>Cumulative new detection§ (95% CI)</th>
<th>No. present at baseline¶</th>
<th>No. detected during follow-up§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cohort</td>
<td>10049</td>
<td>41.0</td>
<td>6</td>
<td>1183/9153 = 12.9% (12.2% to 13.6%)</td>
<td>1020/7811 =13.1% (12.3% to 13.8%)</td>
<td>1468</td>
<td>1420</td>
</tr>
<tr>
<td>Censored at enrollment</td>
<td>955</td>
<td>50.4</td>
<td>n/a</td>
<td>257/915 = 28.1% (25.2% to 31.1%)</td>
<td>n/a</td>
<td>340</td>
<td>n/a</td>
</tr>
<tr>
<td>Actively followed</td>
<td>3065</td>
<td>37.0</td>
<td>7</td>
<td>615/2625 = 23.4% (21.8% to 25.1%)</td>
<td>673/2844 = 23.7% (22.1% to 25.3%)</td>
<td>765</td>
<td>981</td>
</tr>
<tr>
<td>Not sexually active at enrollment</td>
<td>410</td>
<td>21.0</td>
<td>7</td>
<td>n/a</td>
<td>90/256 = 35.2% (29.3% to 41.3%)</td>
<td>n/a</td>
<td>138</td>
</tr>
<tr>
<td>Screen positive</td>
<td>2115</td>
<td>38.4</td>
<td>7</td>
<td>594/2099 = 27.8% (25.9% to 29.8%)</td>
<td>510/2069 = 24.7% (22.8% to 26.6%)</td>
<td>734</td>
<td>749</td>
</tr>
<tr>
<td>Screen negative</td>
<td>540</td>
<td>43.9</td>
<td>7</td>
<td>31/526 = 5.9% (4.0% to 8.3%)</td>
<td>73/519 = 14.1% (11.2% to 17.4%)</td>
<td>31</td>
<td>94</td>
</tr>
<tr>
<td>Passively followed</td>
<td>6029</td>
<td>41.6</td>
<td>6</td>
<td>311/6613 = 5.5% (5.0% to 6.2%)</td>
<td>347/9657 = 7.0% (6.3% to 7.7%)</td>
<td>363</td>
<td>439</td>
</tr>
</tbody>
</table>

* CI = confidence interval; n/a = not applicable; time-bin = 1-y interval where HPV test results were consolidated as one result for each type-specific infection. The following HPV types were considered as carcinogenic: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -68, -59, and -66.
† Proportion of women with at least one carcinogenic HPV infection at enrollment among sexually active women who were screened and had adequate test results.
‡ Proportion of women with new type-specific HPV infections among women who were ever in follow-up, who were sexually active during follow-up, and who were HPV negative at enrollment for that HPV type.
§ Women can contribute one or more type-specific carcinogenic HPV infections at either enrollment and/or follow-up.
¶ Random sample of women who had negative enrollment test results and would have been part of the passive cohort if not selected as a random sample for active follow-up.
†† Women who at enrollment had negative cytology and cervigram results, normal looking cervixes by naked eye inspection, tested negative for HPV DNA by hybrid capture tube test (see "Methods"), and who were screened at enrollment and on average 5.4 y after enrollment.
from sexually active women with a Cervex brush (Unimar, Wilton, CT) at each visit. Additional exfoliated cells were collected using a Dako swab for HPV detection and genotyping; these samples were stored in Virapap DNA transport medium (Digene), or later in the study, the transport medium was shifted to Digene’s DNA specimen transport medium (STM). After collection, the cells in transport medium were kept in coolers at 4°C in the field. They were frozen at −30°C later that day and moved weekly to a −70°C freezer. Periodically, the samples were shipped on dry ice to the National Cancer Institute biorepository in Maryland, where samples were kept in a −70°C mechanical or liquid nitrogen (vapor phase) freezer until tested several years later. After cell collection, the cervix was thoroughly rinsed with 5% acetic acid and two images of the cervix were taken (cervigrams; National Testing Laboratories, Fenton, MO), as described elsewhere (14,15).

HPV Determination by Polymerase Chain Reaction

Specimens from the enrollment visit were tested initially in the United States by HCT, which contributed to the follow-up visit schedule density. However, because this method had limited sensitivity (10 pg/mL) and could only detect 11 of the carcinogenic HPV types, we retested all specimens using a polymerase chain reaction (PCR) test. As previously described (13,22), the amount of DNA extracted from exfoliated cells in a 100-μL aliquot of the STM specimen was amplified using the MY09/MY11 L1 degenerate primer PCR system with AmpliTaq Gold polymerase (TaqGold; Perkin-Elmer-Cetus, Norwalk, CT). After amplification, PCR products were analyzed by electrophoresis and hybridized with radio-labeled generic HPV DNA probes. Dot-blot hybridization was used for HPV typing: Probes were specific for types 2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42–45, 51–59, 61, 62, 64, 66–74, 81–85, and 89. We considered the following HPV types as carcinogenic: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68 (1,23–26). Untyped HPV DNA-positive samples were rare because many HPV-positive specimens were tested twice in different batches. The results of HPV typing were adjudicated by investigators who were masked to clinical outcomes. Approximately 2000 initially negative specimens, including those with and without cytological abnormalities, were retested with additional PCR primers (ie, GP5+ and FAP) to confirm the sensitivity of the main assay. Extremely few additional positive results arose from this confirmatory retesting. All PCR testing and genotyping were done in the United States, at the Albert Einstein College of Medicine in New York.

Cytology and Cervicography

Conventional and liquid-based cytology (Papanicolaou) tests were reported using the Bethesda System (27). Conventional smears were prepared and read in Costa Rica [and also using a computer-assisted screening technology in the United States at enrollment and 1-year follow-up (28)], while liquid-based ThinPreps were interpreted twice, in the United States and, for the last half of follow-up in Costa Rica as well (15). Cervigrams were processed and interpreted by an expert evaluator at National Testing Laboratories Worldwide (15,29).

Colposcopy and Final Diagnostic Group

At enrollment, detection of any screening abnormalities by any test with the exception of the HCT test triggered a referral for colposcopic examination. During follow-up, women were sent for colposcopic evaluation if their screening tests gave us reason to suspect CIN 2 or worse. HPV PCR results were added to the colposcopy referral algorithm only after the last screening visit.

Upon colposcopy, either a punch biopsy or a loop electrosurgical excision procedure was performed based on a woman’s age, parity, colposcopic appearance, and screening results. Women with cancer were referred for further treatment (ie, hysterectomy or radiotherapy) as needed (15). For clinical management, all histology slides were first read in Costa Rica. At the end of follow-up, all slides were reviewed by one of the two pathologists (M. Sherman and D. Solomon) in the United States, who were masked to all other data. The final diagnosis was reached by majority, except for those cases in which all three pathologists disagreed, for which the final diagnosis was achieved after a joint review in the United States. We considered CIN 3 to be the proximate surrogate endpoint for cancer, but, for women’s safety, CIN 2 was the censoring treatment threshold and so we studied the more heterogeneous and less certain diagnosis of CIN 2+ as well (30).

Statistical Analysis

We concentrated only on carcinogenic HPV types because noncarcinogenic types, although interesting in relationship to persistence, are not relevant to cancer development. The proportion of sexually active women who had at least one prevalent carcinogenic HPV infection at enrollment was calculated for the whole cohort and for the three subcohorts: censored, active follow-up, and passive follow-up. Also, we recorded the number and cumulative rate of any new HPV infections (ie, infections among women who were ever in follow-up and who had tested negative at enrollment for that HPV type). The numerators, denominators, and 95% confidence intervals (CIs) are presented for these different proportions. These proportions were also calculated for women in active or passive follow-up for four age groups (18–25, 26–33, 34–41, and ≥42 years). Age groups for a given infection reflected the age at first detection; they were chosen so that about one-quarter of the sexually active women in active follow-up in the cohort were in each group.

The major longitudinal analyses of HPV persistence and of risks of CIN 2+ and CIN 3+ considered the individual history of each carcinogenic HPV infection separately; therefore, one woman could contribute to one or more infections at each time point. We excluded 42 infections from the actively followed subcohort that had uncertain patterns of HPV detection (ie, two or more intervening negative results or uncharacterized types). We categorized infections that were detected at the enrollment visit as “prevalent,” and infections that were detected for the first time after enrollment as “newly detected.” We avoided the term “incident” because it could possibly be misleading: some newly detected infections might be reappearing from an original transmission earlier in a woman’s life. In practical terms, we categorized an infection as “newly detected” if there was at least one previous negative test for that type or if the infection was found in a woman who was a virgin at enrollment. We excluded three infections that were detected
during follow-up among sexually active women for which enrollment results were missing. We considered persistence of each HPV type independently because previous analyses have shown no major interference between types when multiple infections are present (31,32). To standardize time among infections for each infection included in the analysis, each clinic visit was assigned to one of seven time-bins (0, 1, 2, 3, 4, 5, or 6 or more years), with time of first detection of a particular HPV type in a particular patient as time 0. Therefore, for prevalent infections, time-bin 0 equaled the enrollment visit and the age of the woman at the enrollment visit became the age. If the infection was newly detected, the visit where it was detected for the first time became time-bin 0 for that infection and age was set as the age the woman had at the time of that first detection. If an infection was tested more than once in a bin (eg, for those subjects who received accelerated 6-month screening) and if the PCR results were discordant, the overall result was assumed to be positive to acknowledge the possibility of HPV measurement error (76 changes were made). An exception was made for the final (6 or more years bin); in this case, because the time between visits could be much longer than a year, we classified infections as absent or present according to the last measurement.

We compared the proportion of carcinogenic type-specific infections still persisting among infections tested at each time-bin point for prevalent and newly detected infections by the four age groups previously described (Figure 1). The proportion of prevalently detected carcinogenic type-specific infections that persisted until the rescreening visit at years 5–7 in the passive cohort was also calculated for the 6 or more years bin for each of the four age groups (18–25, 26–33, 34–41, and ≥42 years). Because it was possible that a woman could contribute multiple infections to each time point in her age group, generalized estimating equation models with an independent correlation structure and an empirical variance estimate robust to the true correlation structure were used to estimate the proportion of infections that persisted at each time point by age group. We tested equality of proportions using large-sample statistics ($\alpha = .05$, two-tailed) and the chi-squared test for equality of population distributions.

We examined the absolute (cumulative) risk of CIN 2, CIN 3+ (CIN 3 and cancer), and CIN 2+ following HPV detection (predictive value) for each of the same four age groups and screening subcohorts, for prevalent and newly detected infections separately. No HPV determination was made at the tissue level; therefore, in the 11 instances (all within the actively followed group) in which more than one persisting carcinogenic HPV infection was detected at the time of the CIN 2 or CIN 3 histology, we assigned the CIN 2 (n = 2) or CIN 3 (n = 9) to all persisting infections with carcinogenic HPV types (11 pairs). Generalized estimating equation models with an independent correlation structure and an empirical variance estimate robust to the true correlation structure were used to reestimate the cumulative risk of CIN 2, CIN 3, and cancer to adjust for these multiple persisting infections in a same woman diagnosed with CIN 2+ cases.

We observed newly detected infections for a shorter average duration of follow-up than prevalent infections (2.9 vs 5.5 years, respectively). Therefore, to examine predictive values of prevalent vs newly detected infections over a clinically relevant time interval with adequate statistical power, we examined risk of CIN 2 and CIN 3+ for the screening subcohorts at 3 years. To highlight the uniqueness of HPV-16, the risk of CIN 2 and CIN 3+ were estimated for this HPV type separately.

**Results**

The women in the actively and passively followed screening subgroups of the Guanacaste cohort varied in terms of average age and in terms of baseline prevalent carcinogenic HPV infections, as determined by PCR genotyping (Table 1). Women who were censored at enrollment and women who were screen positive and actively followed had similarly high baseline HPV prevalences: 28.1% (95% CI = 25.2% to 31.1%) and 27.8% (95% CI = 23.9% to 29.8%), respectively. The 540 women in the screen-negative sample included in the active cohort and the 6029 women in the passively followed cohort had a similarly low HPV prevalence by PCR because the screen-negative sample was chosen to represent the passive cohort whose members were HCT negative: 5.9% (95% CI = 4.0% to 8.3%) and 5.5% (95% CI = 5.0% to 6.2%), respectively. Newly detected infections were more common among the young women who first became sexually active during active follow-up than in any other subcohort and occurred in 35.2% (95% CI = 29.3% to 41.3%) of these women. As we expected, we detected fewer new infections among the women who tested negative at enrollment and were passively (less frequently) followed than among a smaller group of similar women who were actively followed and had many more visits: 7.0% (95% CI = 6.3% to 7.7%) vs 14.1% (95% CI = 11.2% to 17.4%), respectively.

Prevalence of carcinogenic HPV infections declined with age, except for a small statistically nonsignificant increase in prevalence...
confirmed infections among women aged 42 years and older were observed to have strikingly high viral persistence. However, the absolute risk of CIN 2+ proportion of women with incident CIN 2+ was not immediately found. We found that most (68 of 85) CIN 2 detected in any age group and, interestingly, the percentages of persistently infected women aged 42 years or older showed a much higher proportion of type-specific infections that were detected at baseline for 6 years or more after baseline (Table 3). Approximately 51% of these persistently infected women aged 22–33 years old, 14% of women aged 34–41 years old, and 12.2% among women aged 42 years or older. Again, persistence increased with age, and among women aged 42 years or older showed a much higher proportion of type-specific infections that were persistently detected at baseline for 6 years or more after baseline (Table 3). Approximately 51% of these persistently infected women aged 22–33 years old, 14% of women aged 34–41 years old, and 12.2% among women aged 42 years or older.

We also estimated the average type-specific persistence of carcinogenic HPV infections and, for all infections combined, among women aged 42 years or older showed a much higher proportion of type-specific infections that were persistently detected at baseline for 6 years or more after baseline (Table 3). Approximately 51% of these persistently infected women aged 22–33 years old, 14% of women aged 34–41 years old, and 12.2% among women aged 42 years or older.

Table 2. Prevalent vs newly detected carcinogenic human papillomavirus (HPV) infections among women in the Guanacaste cohort by age within each screening group.

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>No. of women</th>
<th>Median number of follow-up visits</th>
<th>Baseline prevalence* (95% CI)</th>
<th>Cumulative new detection‡ (95% CI)</th>
<th>Carcinogenic HPV infections</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Actively followed§</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of infections</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No. detected during baseline§</td>
</tr>
<tr>
<td>Actively followed‖</td>
<td>3065</td>
<td>6.0</td>
<td>180 (445 = 40.4% (35.9% to 45.2%)</td>
<td>247 (688 = 35.9% (32.3% to 39.6%)</td>
<td>259</td>
</tr>
<tr>
<td>18–25</td>
<td>843</td>
<td>6.0</td>
<td>161 (640 = 25.2% (21.8% to 28.7%)</td>
<td>197 (644 = 30.6% (27.1% to 34.3%)</td>
<td>189</td>
</tr>
<tr>
<td>26–33</td>
<td>664</td>
<td>7.0</td>
<td>90 (660 = 16.1% (13.1% to 19.4%)</td>
<td>100 (554 = 18.1% (14.9% to 21.5%)</td>
<td>106</td>
</tr>
<tr>
<td>34–41</td>
<td>563</td>
<td>7.0</td>
<td>184 (980 = 18.8% (16.4% to 21.4%)</td>
<td>129 (958 = 13.5% (11.4% to 15.8%)</td>
<td>211</td>
</tr>
<tr>
<td>≥42</td>
<td>996</td>
<td>6.0</td>
<td>72 (765 = 9.4% (7.4% to 11.7%)</td>
<td>86 (680 = 12.6% (10.2% to 15.4%)</td>
<td>90</td>
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</table>

* CI = confidence interval. The following HPV types were considered as carcinogenic: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68.
† Proportion of women with at least one carcinogenic HPV infection at enrollment among sexually active women who were screened and had adequate test results.
‡ Proportion of women with new type-specific HPV infections among women who were ever in follow-up, who were sexually active or became sexually active during follow-up, and who were HPV negative at enrollment for that type.
§ Women can contribute one or more type-specific carcinogenic HPV infections at either enrollment and/or follow-up.
‖ Includes 410 women who were not sexually active at enrollment.
¶ Women who at enrollment had negative cytology and cervigram results, normal looking cervixes by naked eye inspection, tested negative for HPV DNA by hybrid capture tube test (see “Methods”), and who were screened at enrollment and on average 5.4 y after enrollment.
two cases of CIN 2+ were detected among the random sample of 540 women who tested negative at enrollment but were nonetheless followed in the active cohort for more than 6 years (data not shown).

Newly detected HPV infections, regardless of the woman’s age at the time of first detection, were associated with very low absolute risk of subsequent CIN 2+ diagnosis. We limited our analysis to the first 3 years of follow-up to compare the clinically relevant absolute risks (which can also be thought of as predictive values) of prevalently vs newly detected infections (Table 4). The relative importance of prevalent infections was accentuated, especially among older women, when we used the more important clinical endpoint of CIN 3+. Risk of CIN 3+ among prevalent infections in women aged 34 years or older was 5.5% compared with 0.5% for newly detected infections. Only one (0.5%) of the 182 newly detected carcinogenic infections among women aged 34 years or older led to a CIN 3+ diagnosis during 3 years of follow-up, whereas 11 (3.5%) of the 312 newly detected carcinogenic infections among women younger than 34 years progressed to CIN 3 (P = .06 for comparisons of two proportions).

Moreover, none of the 17 newly detected HPV-16 infections among women who were 34 years or older (vs 5 [12.2%] of the 41 newly detected HPV-16 infections among women who were younger than 34 years) led to a CIN 3+ diagnosis, whereas four (8.2%) of the 49 prevalently detected HPV-16 infections among women who were 34 years or older (or 8 [15.4%] of the 52 prevalent HPV-16 infections among women who were younger than 34 years) yielded a CIN 3+ diagnosis (data not shown).

**Discussion**

We found that new infections among women at older ages are typically benign, with low absolute risks of persistence, CIN 2+ and especially CIN 3+. The absolute risk and etiologic fraction of...
subsequent cancer, therefore, can be expected to be extremely low. We confirmed that the frequency of new infections, which are the only ones that the current vaccines can prevent (34), decreases with age. Therefore, screening will initially detect old and new infections but sequential rounds will increasingly detect new infections. The formulation of cervical cancer prevention policy among adult women should take into consideration the particular natural history of HPV and cervical neoplasia at those ages.

Longitudinal data for all HPV infections detected during 7 years within the Guanacaste population-based cohort allowed us to directly compare the risk of persistence and progression to CIN 2+ for carcinogenic HPV infections detected prevalently at enrollment (after being persistent for perhaps many years) vs those detected during follow-up (newly detected) in women of several age groups. We confirmed previous findings (16,20,35) that, for prevalently detected infections, the risk of persistence increases with the increasing age of the woman. In women with prevalent infections, subsequent risk of CIN 3+ also increased with age, although the trend in this analysis was not statistically significant for the less robust CIN 2+ endpoint (36). However, newly detected infections, including HPV-16 infections, were able to be cleared as quickly by older women as by younger women, and for newly detected infections, the older women had a similar or slightly decreased risk of CIN 2+ and especially CIN 3 compared with younger women.

This direct age-specific comparison of prevalent and newly detected infections resolved previous reports that had seemed discrepant. Cross-sectional studies in which prevalently detected infections were revisited some years later showed an increasing risk for persistence of infection and/or risk of CIN 2+ with increasing age of the woman (16,36,37). However, analysis of duration of HPV infections detected as “incident” or new during follow-up among women in two other cohorts (18,19) showed that these infections were of similar duration irrespective of the age of the woman, and one-third cohort (38) reported shorter duration among women at older ages. Our data reconcile these findings: The apparent contradiction is resolved by the recognition that, among older women, prevalently and newly detected infections are found at different stages of HPV natural history, with very different implications for prevention programs based on HPV vaccination or HPV testing.

The differences can be understood in the context of the typical “life cycle” of HPV infections, which starts at transmission. The peak transmission of HPV infections usually occurs early in the first years following initiation of sexual intercourse because HPV infections are so endemic and easily transmitted (7,9). The average age of first sexual intercourse tends to center homogeneously on a few years in adolescence and young adulthood. As a corollary, most infections among young women are recently acquired, regardless of whether they are detected prevalently in a cross-sectional screen or in the next few months or years as new. It is not surprising, therefore, that we found no distinction between prevalent and newly detected infections at younger ages in terms of persistence or risk of CIN 2+ in the subsequent 3 years.

As the HPV infection persists, the situation grows more complex. Most infections clear quickly within 6–12 months of detection, but the longer that a carcinogenic HPV infection lasts in a detectable state, the higher the risk that it will continue to persist, or not clear under immunologic control, or lead to a CIN 3+ (31). By the time that such an infection has persisted for approximately 2 or more years, an associated risk of CIN 2 (a part of which is incipient CIN 3) and even CIN 3 is easily noted (11,39). Initially, miniscule CIN 3 lesions can take years to grow to a size that is able to be visualized by colposcopy, biopsied, and diagnosed microscopically (35).

The hazard of CIN 3 rises and falls again as a secondary peak that echoes the earlier peak in HPV infection among younger women. The average time from a carcinogenic HPV infection to clinical diagnosis of CIN 3 (n.b., we are discussing time of diagnosis, not the underlying natural history) is shortened by intensive screening and aggressive management; thus, the CIN 3 that we found associated with prevalent HPV infections tended on average to occur among women almost a decade older than the many fewer cases we found following newly detected infections.

In turn, the incidence of invasive cancers rises as a tertiary wave. Comparison of the average age of cancer diagnosis and the average age of first sexual intercourse within a given population suggests that the typical time interval for a carcinogenic HPV infection to cause CIN 3 and then invasive cervical cancer is greater than 20–25 years. Thus, we observed almost all cancers in the Guanacaste cohort among women who had prevalent long duration infections. There was a very low but nonnull risk of cervical cancer among women who initially tested as HPV negative (40). Notably, these exceptional cases of rapid cancer progression are very important and must be weighed against population averages when screening policy is discussed.

We believe that prevalently detected infections among older women (≥42 years of age in this cohort) include two groups of long-term infections likely to persist longer than new infections: those infections that have an associated CIN 2+ lesion and a residual small fraction of HPV infections that seem to persist for years without evidence of CIN 2+. Although some prevalently detected infections might have been transmitted within the previous few years or might have recently reappeared from an undefined latency, most prevalent detection at older ages corresponds to continually detectable infections of long duration, thereby predicting an elevated risk of further persistence and eventual CIN 2+. Although they were less common, some examples of benign persistence were observed: 25 (2.2%) of the 1128 carcinogenic HPV infections had HPV DNA that was detectable for 7 years, with no evidence of cervical pathology despite repeated multimodal examinations. We also note that there was no evidence that the differences in HPV persistence that we observed among different age groups could be explained by confounding by behavioral covariates, such as parity or oral contraceptive use (data not shown).

Carcinogenic HPV infections that were newly detected upon screening among older women likely represent a heterogeneous set in terms of the real duration or “age” of the infection. Even though it is not possible to distinguish true incidence from temporary loss of immune control of infections initially transmitted years before, the main analysis showed a similar behavior of newly detected infections irrespective of the age of detection, specifically, that there is low risk of subsequent overt HPV persistence and risk of CIN 2+.
As a final point regarding HPV natural history, we confirmed that the infections found only by PCR and not by HCT, that is, the infections with the very lowest viral loads tended to lead to a very low risk of CIN 2+, even though they persisted on average as long as infections with higher viral loads. Most CIN 2+ was caused by HPV-16, and an association between low viral load and low risk of subsequent CIN 2+ is well described for this HPV type but not for others (25,41,42).

The main limitation of this study is the short follow-up time of incident infections. Although this large cohort study evaluated all carcinogenic HPV infections in a true population sample of women aged 18 years and older, the most important conclusion that newly detected HPV infections are benign at any age might not hold beyond the 7 years of follow-up we achieved. We are currently planning a final screening of the cohort at approximately 20 years past enrollment.

Understanding the relevance of the concept of duration of infection can improve cervical cancer prevention strategies that integrate prophylactic vaccination with HPV screening. For example, evidence that newly detected infections in older women do not harbor a higher risk of HPV persistence or CIN 2+ than in younger women and that older women acquire fewer new infections indicates that the possible benefit of vaccinating older women is much reduced, as measured by potential number of averted CIN 2, CIN 3, and cervical cancer diagnoses per 1000 women vaccinated. It is important to add that the sharp peak in prevalent CIN 2+ caused by HPV-16 or HPV-18 that was seen among women around age 30 years upon their enrollment in the Guanacaste cohort likely would have occurred years earlier if these women would have been adequately screened before entering the cohort; thus, vaccination of women in their late 20s or even in their early 20s would not have prevented the majority of these cases.

Screening with HPV must also consider the duration of infections. First-time screening of a population using HPV testing will detect prevalent infections associated with high risk of persistence, CIN 2, and CIN 3, providing excellent risk stratification because HPV-16, and an association between low viral load and low risk of persistence, risk of associated CIN 3, and eventual cervical cancer. Vaccination and screening programs must specify clearly what kind of infections they are targeting to avoid mistaken conclusions.

In conclusion, more than 90% of new infections with carcinogenic HPV types are benign, whereas long duration of infection predicts risk of further persistence, risk of associated CIN 3, and eventual cervical cancer. Vaccination and screening programs must specify clearly what kind of infections they are targeting to avoid mistaken conclusions.

References


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**Notes**

The authors take full responsibility for the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

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