Human Papillomavirus Infection and Time to Progression and Regression of Cervical Intraepithelial Neoplasia

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Background: Little is known about the duration of precancerous cervical lesions in relation to human papillomavirus (HPV) infection. We estimated rates of progression and regression and sojourn times of cervical squamous intraepithelial lesions (SILs) according to HPV status. Methods: We used data from a longitudinal study of HPV infection and cervical neoplasia in São Paulo, Brazil. Cervical specimens were taken from 2404 women for Pap cytology and polymerase chain reaction–based HPV testing every 4–6 months over a period of 8 years. We used actuarial and non-actuarial analyses to measure time to and rates of lesion progression and regression according to status and type of HPV infection. Results: During follow-up, 118 low-grade SIL (LSIL), 24 high-grade SIL (HSIL), and 173 atypical squamous cells of undetermined significance (ASCUS) events were detected. Mean time to progression from ASCUS to LSIL or worse and from LSIL to HSIL or worse was shorter in women with oncogenic HPV types than in women with no HPV infection (mean times for ASCUS progression were 67.0 and 88.0 months, respectively, in women with oncogenic HPV and no HPV, difference = 21.0 months, 95% confidence interval [CI] = 11.3 to 30.7 months; mean times for LSIL progression were 73.3 and 83.5 months, respectively, difference = 10.2 months, 95% CI = –0.15 to 20.6 months). Half of the LSILs regressed to normal or ASCUS within 6 months. Mean times for progression from ASCUS to normal, from LSIL to ASCUS or normal, and from HSIL/cervical intraepithelial neoplasia 2 to ASCUS or normal were longer for women with oncogenic HPV types (16.8 months, 95% CI = 7.5 to 26.2 months; 13.8 months, 95% CI = 8.8 to 18.7 months; and 17.1 months, 95% CI = 4.1 to 30.1 months, respectively) than for women with non-oncogenic HPV types (7.7 months, 95% CI = 5.2 to 10.2 months; 7.8 months, 95% CI = 5.3 to 10.2 months; 8.9 months, 95% CI = 3.3 to 14.6 months) or for women with no HPV infection (7.6 months, 95% CI = 6.9 to 8.4 months; 7.6 months, 95% CI = 6.4 to 8.7 months; and 7.0 months, 95% CI = 5.0 to 8.9 months, respectively). Conclusion: Precursor lesions of the cervix persist longer and progress more quickly in women with oncogenic HPV infections than in women with non-oncogenic infections or without HPV. Testing cervical lesions for oncogenic HPVs may help identify those that are likely to progress rapidly. [J Natl Cancer Inst 2003;95:1336–43]

The natural history of cervical cancer involves reversible changes in the cervical tissue from a normal state, in which no neoplastic changes are detected in the squamous epithelium, to varying states of cellular abnormalities that ultimately lead to cervical cancer (1). This sequence forms the premise on which cytologic screening for cervical cancer is based and corresponds to an underlying multistep carcinogenic process in the development of cervical intraepithelial neoplasia (CIN) (2). Low-grade squamous intraepithelial lesions (LSILs) may progress to high-grade SILs (HSILs) and invasive cervical cancer or may regress to a normal state (3). However, few studies of cervical neoplasia have evaluated lesion recurrence (4,5) or disease progression (3) over time. Likewise, lesion progression or regression has not been evaluated in relation to the presence of human papillomaviruses (HPV), the main etiologic agents in the initiation of cervical neoplasia (6). The use of a biomarker that can predict the rate of progression or regression and the duration of the preinvasive stages of cervical cancer could represent an attractive means for targeting screening or chemoprevention.

Beginning in 1993, we initiated a cohort study involving repeated measurements of HPV infection and cervical cytology in women attending a comprehensive maternal and child health program that serves low-income families living in neighborhoods located in the northern sector of the city of São Paulo, Brazil (7). In this population, early precursor lesions are generally not treated, which enabled us to evaluate prospectively the occurrence of SIL events at regular intervals over time. In particular, we sought to measure the frequency and rates of progression and regression, as well as the durations (i.e., sojourn time) of early cervical precursor lesions according to their HPV status.

SUBJECTS AND METHODS

Subject Recruitment

Two study nurses approached 4990 women from daily lists of outpatients in the family medicine, gynecology, and family planning clinics at the Vila Nova Cachoeirinha municipal hospital in São Paulo, Brazil, for an interview. Women who were poten-
Women were eligible to participate if they 1) were between 18 and 60 years of age; 2) were permanent residents of São Paulo (city), Brazil; 3) were not currently pregnant and had no intention of becoming pregnant during the next 12 months; 4) had an intact uterus and no current referral for hysterectomy; 5) reported no use of vaginal medication in the previous 2 days; and 6) had not received treatment for cervical disease in the previous 6 months. In addition to these criteria, women were considered eligible only if they expressed willingness to comply with all scheduled return visits, at least for the initial 2 years.

Subjects entered the study only after giving signed informed consent. The study protocol was approved by institutional ethical and research review boards of the participating institutions in Canada (McGill University) and Brazil (Ludwig Institute for Cancer Research). A detailed description of the design and methods of the study has been published previously (7). All participants were seen every 4 months in the first year (at 0, 4, 8, and 12 months) and every 6 months thereafter. Delays in returning for a given follow-up appointment were allowed; the visit numbering sequence was maintained, even when subjects returned for their follow-up visit after the scheduled date, with the information and specimens collected being assigned to the originally scheduled follow-up visit. As a result, the same number of scheduled visits was retained, precluding missing interval visits. However, all time-to-event analyses were based on the actual time of the visits. Cervical specimens were taken for conventional Pap smear and HPV testing at every visit. The study nurses also performed a detailed interview at enrollment to collect information on sociodemographic factors, reproductive health, sexual activity, and smoking status. Information on sexual activity and reproductive health was also collected at each return visit during the first 12 months and annually thereafter.

Cervical Cell Specimens

An Accelon biosampler (Medscand, Hollywood, FL) was used to collect ecto- and endocervical samples. After cells were smeared on a glass slide and fixed for Pap cytology, the sampler containing the residual exfoliated cells was immersed in a tube containing Tris–EDTA buffer (pH 7.4) (7). The exfoliated cell samples were sent to the Ludwig Institute for Cancer Research in São Paulo for storage and HPV testing. The Pap smears were shipped to McGill University for coding and classification by an expert cytopathologist (A. Ferenczy) who was blinded to previous cytology outcomes and to HPV results for the same and previous samples. The cytopathology reports were based on the 1991 Bethesda system for cytologic diagnoses (8). Referral to colposcopy was made by the Study Management Center at McGill University on the basis of either local or review cytology reports or the cervicography examination, which was performed once every 2 years for all women.

The progression and regression states were classified by lesion severity (LSIL or HSIL) and were subclassified within these lesion grades. LSILs were separated into those with koilocyosis (LSIL/HPV) and those without koilocyosis but with squamous abnormalities (LSIL/SQ). Similarly, HSILs were separated into those indicative of CIN2 (HSIL/CIN2) and those indicative of CIN3 (HSIL/CIN3).

HPV DNA Detection

Cervical specimens were tested for the presence of HPV DNA by using the MY09/11 polymerase chain reaction (PCR) protocol (9,10). The amplified products were typed by hybridization with individual oligonucleotide probes specific for 27 genital HPV types (10). Amplified products that hybridized with the generic probe but with none of the type-specific probes were further tested by restriction fragment length polymorphism analysis (11) to extend the range of identifiable HPV types. To verify the specificity of the hybridizations, we included more than 30 type-specific positive controls in each HPV test run. To check the integrity of the host DNA extracted from the specimens, assays also included an additional set of primers to amplify the β-globin gene (9). HPV types were separated into two groups by level of oncogenicity. Oncogenic types included HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68; non-oncogenic types included 6/11, -26, -34, -40, -42, -44, -53, -54, -55, -57, -62, -64, -66, -67, -69, -70, -71, -72, -73, -81, -82, -83, -84, CP6108, and other unknown types. All HPV assays were done on coded specimens, with no identification that could link specimens from the same woman. Appropriate precautions were taken to reduce the possibility of specimen contamination.

Statistical Analyses

For the analyses reported here, follow-up began in November 1993 and continued through mid-April 2002. Subjects with atypical squamous cells of undetermined significance (ASCUS), LSIL, or HSIL on cytology were included in each risk set at the time of their first detected result. Depending on the index lesion of interest, prevalent cases of equivalent grade of abnormality at enrollment were excluded from the risk set at baseline. Subjects who were biopsied were censored at the time of their biopsy if no transition event had occurred before the biopsy date to reduce the potential for interference by the biopsy procedure on estimates of time to regression. Women who dropped out of the study were censored at their last visit date. In time-to-event analyses, time to event was measured from the date of the index visit (i.e., the first instance of an abnormal cytologic result) to the date of the visit at which transition to a more or less severe cytologic category (for estimates of progression or regression, respectively) was first detected or, for censored subjects, to the last recorded return visit date. Such data represent interval-censored data because the exact dates of HPV infection and SIL incidence are not known. The time to regression from HSIL or LSIL was defined as the time from the index visit until the first follow-up visit at which a subject presented with LSIL, ASCUS, or a normal Pap smear (depending on the regression endpoint of interest), whether or not a worse cytologic event had been detected during intervening visits. Times to progression or regression were evaluated for the overall group at risk by age and ethnic group and for groups stratified by HPV status at the visit when the index lesion was first detected or at the visit closest to the index event at which a valid HPV result was obtained. Oncogenic status of HPV in the index specimen was coded according to the following hierarchic categories: 1) no HPV detected, 2) only non-oncogenic types detected in the index specimen, and 3) any oncogenic HPV type detected.

The cumulative probability of a lesion’s remaining in the same stage or progressing to the next stage was estimated by...
actuarial analysis using Kaplan–Meier curves (12) as a function of the length of follow-up, stratified by HPV status at the index visit. The life table method was also used to estimate the proportion of women who remained positive for a precursor lesion during follow-up without progressing to a higher stage, according to HPV status at the index visit (13). Ninety-five percent confidence intervals (CIs) for the actuarial estimates were calculated using the standard error of the cumulative probability at the end of a particular interval in which an event occurred (14) or, for binomial proportions, via exact estimation. Statistical comparisons (two-sided tests) of lesion sojourn times according to HPV status were performed by using the log-rank test. Progression and regression density estimates were also calculated by dividing the number of incident events (of progression or regression, respectively) by the length of follow-up in person-months of subjects at risk (i.e., those with the index lesion event). Density estimates were likewise stratified by HPV status of the index lesion. Density rates and mean durations were compared by pooled variance z tests and t tests, respectively. Statistical analyses were performed by using SPSS version 11.0 (SPSS, Chicago, IL) and PEPI version 4.0× (Sagebrush Press, Salt Lake City, UT).

For non-actuarial estimates of mean lesion duration, we used a standard formula based on the epidemiologic tenet that, within a stationary population and in the absence of migration, the prevalence proportion (P) is a function of the incidence rate (I) and of the mean duration (D) of the condition. Therefore, the average duration can be estimated with the general formula \[ D = \frac{P}{I \times (1 - P)} \], where \( P \) is calculated as a weighted average of the point prevalence over time for each lesion grade. This formula holds provided that the point prevalence within each stratum is less than 10% (15), a condition that was met for all of the individual lesion grades analyzed in this study. The non-actuarial estimates were stratified by the cumulative HPV status over the first year of follow-up, taking into account test results from the first four visits.

**Results**

Of the 4990 women initially identified, 3589 women initially met the eligibility criteria and were invited to participate in the study. Between November 1993 and March 1997, 2528 women were enrolled in the study, a response rate of 70.4%. A further 66 women were found not to fit the eligibility criteria after enrollment. Fifty-one women (2.1%) developed LSIL and 24 developed HSIL over the period of follow-up. Between November 1993 and March 1997, 2528 women met the eligibility criteria and were invited to participate in the study. Over the first year of follow-up, taking into account test results from the first four visits, the average interval between enrollment was 128,129 person-months, for a mean follow-up duration of 53.3 months per subject. The average interval between visits was 4.8 months for the first year and 6.8 months for subsequent years. Although visits were scheduled according to the study design to occur every 6 months after the first year, the actual intervals between visits in the second and subsequent years ranged from 2.9 months to 81.3 months.

The majority of ASCUS (147/173) and LSIL (104/118) events detected by cytology regressed to a lower grade during follow-up (Table 1). Actuarial analyses showed that half of these abnormalities regressed within 6 months of first detection. The overall mean time to regression of ASCUS abnormalities was shorter than that of LSILs. The mean time to regression from LSIL to ASCUS or normal was generally longer for lesions with oncogenic HPV types (13.8 months) than for lesions with non-oncogenic HPV types (7.8 months) (difference = 6.0 months, 95% CI = −0.7 to 12.7 months) or for HPV-negative lesions (7.6 months) (difference = 6.2 months, 95% CI = 1.0 to 11.4 months, respectively). Median durations, however (i.e., 6.1, 5.3, and 6.0 months for LSILs with oncogenic HPV types, with non-oncogenic HPV types, and without HPV, respectively), differed less than the respective means. Similar differences in regression times within levels of HPV status were observed for the subcategories of LSIL.

To evaluate mean sojourn times with respect to cumulative HPV status during the first year of follow-up, we also calculated mean lesion duration based on a non-actuarial formula (see the “Subjects and Methods” section) in which subjects were stratified by cumulative HPV status during the first year of follow-up. Given the particular relevance of HPV16 in cervical cancer etiology, subjects who tested positive for that type in any of the visits were evaluated separately from those with other oncogenic HPV types. The mean ASCUS duration in women who were HPV-negative at all four visits was 7.9 months, which was lower than that in women with only non-oncogenic HPV types (10.5 months), in women with any oncogenic HPV type (excluding HPV16 at any visit [15.4 months]), and in women positive for HPV16 at any visit (13.4 months). The equivalent mean LSIL durations were 8.9, 10.3, 12.2, and 13.4 months for HPV-negative women and women with non-oncogenic HPV types, oncogenic HPV types, and HPV16, respectively. The equivalent mean HSIL durations were 7.6, 5.7, 15.6, and 57.0 months, respectively.

With respect to progression to a higher preinvasive lesion grade, we observed the reverse of the pattern described above for regression by HPV infection status (Table 2). That is, lesions in subjects with no HPV detected in the index specimen took longer to progress than those with oncogenic HPV. For example, mean time to progression from ASCUS to LSIL or worse and from LSIL to HSIL was shorter for women with oncogenic HPV types than for women with no HPV infection (for ASCUS progression, means = 67.0 and 88.0 months, respectively; difference = 21.0 months [95% CI = 11.3 to 30.7 months]; for LSIL progression, means = 73.3 and 83.5 months, respectively; difference = 10.2 months [95% CI = −0.15 to 20.6 months]) or in women with non-oncogenic HPV types, with respect to LSIL to HSIL progression. Among women with persistent LSIL (n = 24, data not shown), 20% progressed to HSIL or cervical cancer during follow-up.
To evaluate whether lesions progressed more quickly with age, we estimated rates of progression separately for younger and older women. On average, women aged 31–65 years progressed to HSIL from an incident LSIL more rapidly (mean time to progression = 77.9 months) than women aged 16–30 years (mean time to progression = 88.4 months, difference = 10.5 months [95% CI = 1.5 to 19.5 months]). By contrast, the mean time to progression from ASCUS to HSIL was shorter in younger women (81.8 months) than in older women (90.4 months, difference = 8.6 months [95% CI = 4.0 to 13.2 months]). After stratification by HPV status, older women with oncogenic HPV types, including HPV16, had a higher cumulative risk of progression to HSIL than younger women, regardless of baseline abnormality (Fig. 1), although the differences were not statistically significant. Mean times to progression from ASCUS to HSIL or cancer for women with oncogenic HPV infections were 73.4 months and 80.4 months in older and younger women, respectively (difference = 7.0 months, 95% CI = –10.2 to 24.2 months).

We also evaluated rates of progression and regression for subjects with respect to ethnic origin (data not shown). The regression density rates of incident ASCUS, LSIL, and HSIL to normal for whites (n = 1542) and nonwhites (n = 856) were not statistically significantly different (differences in lesion regression rates = 0.61 regressed abnormalities per 100 person-months [95% CI = –1.00 to 11.2]; 2.4 per 100 person-months [95% CI = –9.8 to 14.6]; and 4.7 per 100 person-months [95% CI = –66.7 to 76.1], respectively). Similarly, we saw little difference in rates of progression to HSIL or worse between ethnic groups.

Given the potential for biopsy interventions to interfere with the natural history of HSIL, we estimated the time to regression for HSILs for women based on whether their biopsies were performed before or after a regression event to ASCUS or normal.
Table 2. Actuarial estimates and 95% confidence intervals (CIs) of time to progression of first incident cervical abnormality events stratified by human papillomavirus (HPV) status in index lesions*

<table>
<thead>
<tr>
<th>Index lesion and progression event, HPV status in index lesion†</th>
<th>No. of events/total</th>
<th>Person-months of follow-up</th>
<th>Progression density rate‡ (95% CI)</th>
<th>Mean time to progression, mo (95% CI)</th>
<th>Proportion§ progressing to worse abnormality (95% CI) at 6 mo</th>
<th>12 mo</th>
<th>18 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCUS to LSIL or worse</td>
<td>18/174</td>
<td>6867.3</td>
<td>0.3 (0.2 to 0.4)</td>
<td>82.7 (78.4 to 87.1)</td>
<td>3.7 (0.8 to 6.6)</td>
<td>7.9 (3.6 to 12.2)</td>
<td>8.6 (4.1 to 13.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>6/117</td>
<td>4992.2</td>
<td>0.12 (0.05 to 0.2)</td>
<td>88.0 (84.3 to 91.7)</td>
<td>0.9 (0.0 to 2.7)</td>
<td>3.9 (0.2 to 7.6)</td>
<td>3.9 (0.2 to 7.6)</td>
</tr>
<tr>
<td>Non-oncogenic</td>
<td>5/24</td>
<td>756.5</td>
<td>0.7 (0.2 to 1.5)</td>
<td>67.9 (59.1 to 83.9)</td>
<td>9.8 (0.0 to 22.7)</td>
<td>21.5 (2.7 to 40.3)</td>
<td>21.5 (2.7 to 40.3)</td>
</tr>
<tr>
<td>Oncogenic#</td>
<td>7/31</td>
<td>1112.8</td>
<td>0.63 (0.3 to 1.2)</td>
<td>67.0 (54.6 to 79.5)</td>
<td>10.3 (0.0 to 21.5)</td>
<td>14.2 (1.3 to 27.1)</td>
<td>18.2 (3.7 to 32.7)</td>
</tr>
<tr>
<td>LSIL (any) to HSIL or worse</td>
<td>11/24</td>
<td>5365.5</td>
<td>0.2 (0.1 to 0.4)</td>
<td>85.7 (80.8 to 90.6)</td>
<td>1.7 (0.0 to 4.1)</td>
<td>3.6 (0.1 to 7.1)</td>
<td>6.5 (1.8 to 11.2)</td>
</tr>
<tr>
<td>Negative</td>
<td>4/12</td>
<td>1676.3</td>
<td>0.12 (0.02 to 0.4)</td>
<td>83.5 (78.0 to 89.1)</td>
<td>0.0 (0.0 to 6.6)</td>
<td>0.0 (0.0 to 7.0)</td>
<td>2.8 (0.0 to 8.3)</td>
</tr>
<tr>
<td>Non-oncogenic</td>
<td>1/28</td>
<td>1563.8</td>
<td>0.06 (0.0 to 0.3)</td>
<td>91.3 (85.1 to 97.4)</td>
<td>0.0 (0.0 to 10.1)</td>
<td>3.6 (0.0 to 10.7)</td>
<td>3.6 (0.0 to 10.7)</td>
</tr>
<tr>
<td>Oncogenic#</td>
<td>8/45</td>
<td>2107.7</td>
<td>0.36 (0.2 to 0.7)</td>
<td>73.3 (64.8 to 81.8)</td>
<td>4.6 (0.0 to 10.7)</td>
<td>6.9 (0.0 to 14.5)</td>
<td>11.9 (2.1 to 21.7)</td>
</tr>
<tr>
<td>LSIL/SQ to HSIL/CIN3 Overall*</td>
<td>4/72</td>
<td>3482.6</td>
<td>0.11 (0.04 to 0.3)</td>
<td>89.2 (84.2 to 94.2)</td>
<td>1.0 (0.0 to 3.0)</td>
<td>1.4 (0.0 to 4.1)</td>
<td>3.0 (0.0 to 7.1)</td>
</tr>
<tr>
<td>LSIL/HPV to HSIL/CIN3 Overall**</td>
<td>2/56</td>
<td>2266.5</td>
<td>0.09 (0.01 to 0.3)</td>
<td>86.4 (81.9 to 90.9)</td>
<td>1.8 (0.0 to 5.3)</td>
<td>1.8 (0.0 to 5.3)</td>
<td>1.8 (0.0 to 5.3)</td>
</tr>
</tbody>
</table>

*ASCII = atypical squamous cells of undetermined significance; LSIL/SQ = low-grade squamous intra-epithelial lesion (LSIL) showing squamous effects equivalent to cervical intra-epithelial neoplasia grade 1; LSIL/HPV = LSIL with koilocytotic atypia induced by a productive HPV infection; HSIL/CIN3 = high-grade squamous intra-epithelial lesion (HSIL) with severe dysplasia equivalent to cervical intra-epithelial neoplasia grade 3.
†Index lesion is defined as the first detected event of the stated cytologic abnormality.
‡Number of lesions progressed/total number of index lesions. Number of HPV stratum-specific samples may not add up to the overall number if valid HPV results were unavailable for some samples.
§Progression density rate is equal to the number of incident events per 100 person-months.
∥Proportion of subjects progressing to worse abnormality at end of interval period (6, 12, or 18 months) derived by life table analysis.
#Any oncogenic HPV types in baseline smear.
**All baseline (index) LSIL events were oncogenic HPV-positive.

Discussion

To date, few studies have investigated rates of progression and regression of preinvasive cervical lesions in relation to cervical cancer risk factors (2,3,16–18). The available evidence on the natural history of HPV and cervical neoplasia suggests that a relationship between the likelihood of precursor lesions persisting and progressing to cancer is dependent on the characteristics of the HPV infection (4,6,19,20). In the current longitudinal study, we found that precursor lesions of the cervix detected by cytology persisted longer and were more likely to progress in women with oncogenic HPV infections than in women with non-oncogenic HPV infections or in uninfected women. Using repeated screening by cytology, we were able to evaluate the progression and regression of cervical lesions over time in a more systematic fashion than that in previous studies, which involved registry data and screening programs based on passive data collection.

Our study does have several limitations. First, given that our outcome ascertainment was based on cytologic analysis, one potential limitation is misclassification of lesion outcome history, even though the cytologic assessments were carefully conducted in a reference laboratory following a strict quality-control protocol. We opted for an intensive, expert cytologic review of all subjects in the study every 4–6 months and referred all instances of HSILs for colposcopy. This approach reduced the likelihood of unnecessary biopsies, which can interfere with the natural history of early lesions (21). Nevertheless, the occurrence of false-negative Pap tests could have resulted in underestimates (or shorter estimates) of regression time and in either overestimates or underestimates of progression time, depending on whether these test results occurred at lesion outset or during the sojourn period. In a prospective study of histologically diagnosed carcinoma in situ (CIS), McIndoe et al. (16) found that...
54% of the cases had normal cytology during screening after a punch biopsy, with only one case of six developing invasive carcinoma within 4 years. We therefore censored subjects at the time of their biopsy, anticipating that the procedure could influence the rates of disease determined by cytology.

Studies using both histology and cytology to follow the natural history of cervical neoplasia have shown no effect of limited sampling by punch biopsy on the short-term course of dysplasia (22). In a review of 27 studies of CIN, Mitchell et al. (2) observed similar probabilities of regression, persistence, and progression based on biopsy evaluation and cytology. We found that HSIL persisted for an average of 5.6 months following a biopsy. Although persistent lesions are more likely to be biopsied before regression than lesions of short duration, we found no evidence that biopsy affected the persistence of HSILs, at least in the short term. Although more aggressive standards for biopsy were adopted after a number of years into the study by the local colposcopists (all women referred for colposcopy are currently obtaining biopsies following our recommendations), we cannot exclude the possibility that the biopsy procedures performed earlier in the study were preferentially done for lesions that appeared more severe on colposcopy. Such lesions may have taken longer to regress, regardless of whether a biopsy had been done.

Another possible limitation was misclassification of HPV status. Misclassification was unlikely because we used a highly sensitive PCR-based testing method. The estimates of HPV positivity by cytologic category in the present study are not substantially different from other published estimates for ASCUS and LSIL patients (23), which reflects positively on the quality of the cervical specimens taken, as well as on the sensitivity of the PCR-based method we used for HPV DNA detection. In this analysis, we chose to use the HPV DNA test results obtained from the same specimen that was used for Pap cytology. This decision was based on the assumption that finding HPV in incident lesions is a proxy for prior HPV infection states that led to the lesion. Cross-sectional assessment of HPV and lesion status precludes the determination of directionality in the association between infection and lesion development. However, this approach was chosen to generate data similar to those obtained in screening and on which triage decisions are usually based.

We used two methods to calculate the mean duration or sojourn time of incident cytologic lesions: an actuarial method and a non-actuarial formula—the prevalence–incidence relation. Estimates of mean duration based on actuarial probability estimates indicated that lesions with oncogenic HPV infections persisted longer than lesions with non-oncogenic HPV infections or than lesions without HPV infection. In general, the average duration estimates based on the non-actuarial formula were similar to those obtained with the actuarial method. However, the mean duration of HSIL estimated by the non-actuarial formula was longer than the equivalent estimate by actuarial analysis for subjects with HPV16. The non-actuarial prevalence–incidence relation method is appropriate for estimating average duration of...
incident conditions, such as cytologic abnormalities consistent with ASCUS, LSIL, or HSIL that rarely exceed a prevalence of 10% in most clinical settings (15). However, the non-actuarial formula does not account for censored data (i.e., incomplete observations due to lesions that have not cleared at the study closing date or losses to follow-up). By contrast, actuarial estimates of time to progression were restricted to the longest follow-up time available. As a result, mean times were underestimated when the largest observed analysis time occurred for a censored subject. This effect was greater for strata with smaller numbers of observations. Therefore, we did not separate out the subjects with HPV16 in the actuarial analyses. This difference in estimates from the actuarial and non-actuarial analyses underscores the fact that, due to the heterogeneity of follow-up times and censoring, no single statistical summary measure is appropriate to capture the average progression or regression times of lesions in a repeated-measurements cohort study. Whenever appropriate, therefore, we reported all three summary statistics (non-actuarial means, actuarial means, and actuarial medians) to provide a more complete picture of transit times.

It is conceivable that using shorter cytologic screening intervals could have resulted in more precise estimates of the duration of lesion sojourn times. Indeed, the reduction in follow-up frequency to every 6 months after the first year (when return visits were scheduled at 4 months) may have increased the observed sojourn time. However, this effect would have been equal across HPV groups because all cytology and HPV evaluations were carried out blindly with respect to previous results for the same subject, making comparisons valid on a relative scale.

Several reviews and meta-analyses have attempted to summarize rates of progression and regression along the continuum of cervical neoplastic changes. Östör (17) reported decreasing probabilities of regression for different CIN grades of increasing severity detected by histology. Mitchell et al. (2) observed probabilities of regression, persistence, and progression of all grades of CIN combined to any higher grade lesion of 34%, 41%, and 25%, respectively. Melnikov et al. (18) calculated the following weighted average rates of progression to HSIL, at 24 months according to baseline cytologic abnormality: 7.1%, 20.8%, and 23.4% for ASCUS, LSIL, and HSIL, persistence, respectively. Conversely, average rates of regression to a normal Pap smear were 68.2% for ASCUS, 47.4% for LSIL, and 35.0% for HSIL. Using mild dysplasia as the referent category, Holowaty et al. (3) found relative risks of CIS of 8.1 (95% CI = 6.2 to 10.7) for women with moderate dysplasia and 22.7 (95% CI = 16.0 to 32.1) for those with severe dysplasia within a 2-year period. They also observed that rates of progression were higher during the first 2 years following a positive Pap smear than in subsequent years. This increase was interpreted to be the result of underrating the original smear. Their recommendation, therefore, was to repeat smears within 6 months of the first positive smear rather than 1 year later. We propose a less aggressive recommendation because one-half to one-third of all LSIL and HSIL/CIN2 lesions in our study regressed to ASCUS or normal within 6 months. Repeat screening with a shorter delay would therefore detect a substantial proportion of lesions that would regress spontaneously. Furthermore, given a mean time to progression for LSILs of 85.7 months, most repeat cytology screenings before 1 year would not indicate whether a lesion is likely to progress.

Of the reviews indicated above, few evaluated progression or regression by risk stratifiers. Holowaty et al. (3) examined the influence of parity, age, oral contraceptive use, and number of positive smears on the relative risk of progression of LSILs. They found no relationship between these factors and the relative risk of progression. Similarly, in our study, the mean time to progression was not statistically significantly different between age groups, although we found that, among women with high-risk oncogenic HPV types, progression rates were higher in women 31 years and older than in younger women. These findings support the World Health Organization’s recommendation for focusing screening on older women at risk for cervical cancer (24,25). We also observed shorter progression transit times from ASCUS to LSIL and worse in women with non-oncogenic (low-risk) and oncogenic (high-risk) HPV types compared with women who had HPV-negative abnormalities. This observation provides further evidence of the importance of colposcopic evaluation of women with HPV-positive ASCUS smears, particularly those with high-risk oncogenic HPV types (23).

In conclusion, using screening tests for oncogenic HPVs may help identify those lesions that are likely to progress quickly to more advanced stages. HPV testing of women with abnormal Pap smears may therefore help identify women who might benefit from colposcopic evaluation and, if appropriate, chemopreventive treatment. The ability to identify subjects whose lesions are likely to take longer to progress could also be cost-saving by increasing the follow-up intervals and reducing the morbidity which may result from potentially unnecessary invasive diagnostic and therapeutic procedures.

**REFERENCES**


NOTES

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