Follow-up Outcomes in a Large Cohort of Patients With HPV-Negative LSIL Cervical Screening Test Results

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ABSTRACT

Objectives: Cervical screening guidelines now recommend repeat cotesting of patients aged 30 years and older having a human papillomavirus (HPV)–negative low-grade squamous intraepithelial lesion (LSIL) in 1 year as preferred management. Only limited follow-up data on patients with HPV-negative LSILs are available from routine US clinical practice settings.

Methods: In total, 680 patients with Hybrid Capture 2 (Qiagen, Hilden, Germany) high-risk HPV-negative LSIL ThinPrep (Hologic, Marlborough, MA) results were identified. Patients’ ages and histopathologic, cytologic, and HPV follow-up results were identified.

Results: Among 680 patients with HPV-negative LSILs, 468 had follow-up within 1 year. During the study period, 14 (3.0%) of 468 had follow-up high-grade squamous intraepithelial lesion (HSIL) and 184 (39.3%) LSIL findings. No diagnoses of cervical carcinoma were documented. There were no significant follow-up differences between age groups. Of the 321 patients who had follow-up HPV testing, 271 (84.4%) had negative and 50 (15.6%) had positive HPV results.

Conclusions: This is the largest study documenting follow-up results for patients with HPV-negative LSIL results based on prevalent US FDA–approved cotesting methods from one collection vial. These data document that risk for follow-up HSILs in these patients is low and also that no cervical cancers were diagnosed. These findings support recent recommendations for repeat cotesting after 1 year as an appropriate option for patients with HPV-negative LSIL results.

Low-grade squamous intraepithelial lesion (LSIL) is a cytologic category recognized in The Bethesda System (TBS) that is associated with cytologic evidence of human papillomavirus (HPV) infection. It has been reported that 55% to 89% of LSIL Papanicolaou (Pap) test results will test high-risk HPV (hrHPV) positive, with the influential Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) documenting 83% of LSIL cases as hrHPV positive. HPV-positive rates with LSILs have been shown to be significantly higher in younger populations, such as in ALTS, and lower in older populations. Risk for a follow-up diagnosis of high-grade cervical intraepithelial neoplasia grade 2 and above (CIN2+) in HPV-positive patients with LSILs has ranged from 13% to 19%. Early findings in
ALTS of HPV-positive rates more than 80% and significant risk for histopathologic CIN2+ diagnoses with LSILs led to consensus that there was little utility for routine reflex HPV testing in the management of LSIL Pap test results. Therefore, in 2001, the American Society for Colposcopy and Cervical Pathology Consensus Guidelines and the American College of Obstetricians and Gynecologists recommended immediate colposcopy for all nonadolescent premenopausal women with LSILs, regardless of HPV results.11

Subsequent publication of ALTS data confirmed that 5% to 16% of women with LSIL Pap results tested hrHPV negative.7 Several explanations for the presence of hrHPV-negative LSILs have been put forward, including nononcogenic (low-risk) HPV infections, intentional cut point–related sensitivity limitations for hrHPV tests to enhance specificity, false-positive Pap test results, and viral clearance before the time of testing.5-7 Recent data have further demonstrated that the risk for histopathologic CIN2+ after HPV-negative LSIL results may be as low as 2% to 5%, significantly less than for patients with hrHPV-positive LSILs.5-7,12 These findings have led to the concept that women with HPV-negative LSIL cotest results might be better managed less aggressively. As a result, more recent cervical screening test management guidelines recommend that preferred follow-up of HPV-negative LSILs is repeat cytologic and hrHPV cotesting in 1 year, deferring immediate elective colposcopy for many.13

In this study, we document routine US clinical practice follow-up results for a large cohort of patients with HPV-negative LSIL results based on liquid-based cytology (LBC) findings and US Food and Drug Administration (FDA)–approved hrHPV cotesting from the same preservative vial. We examined the rate of follow-up high-grade squamous intraepithelial lesion (HSIL) findings within 1 year after HPV-negative LSIL findings and also through the extended duration of the full study period to further evaluate the level of risk associated with recent cervical screening test management guidelines.

Materials and Methods

Case Selection

A retrospective study was designed and initiated after obtaining approval from the institutional review board at the University of Pittsburgh Medical Center (UPMC). A computer-based search was carried out on our CoPath laboratory information system to retrieve Pap tests with LSIL interpretations with a concurrent negative hrHPV DNA testing period between June 1, 2005, and June 30, 2013. All specimens were processed in the Magee-Womens Hospital (MWH) of the UPMC cytopathology laboratory and were reported using the 2001 TBS criteria and terminology.1 The MWH of the UPMC cytopathology laboratory is a large, subspecialized academic hospital laboratory that consistently reports more than 100,000 cervical screening tests per year from a large, integrated hospital health system and serves a metropolitan area with a significantly older age profile than the national average.14 The Pap and HPV testing-related reporting profile of our laboratory has been documented in numerous recent publications.15-32

Cytologic Methods

ThinPrep Pap tests (TPPTs; Hologic, Marlborough, MA) were prepared according to the manufacturer’s specifications from PreservCyt samples using an automated processor (ThinPrep 3000). Staining of the slides was done on a Sakura Tissue-Tek Automated Slide Stainer (Sakura Finetek USA, Torrance, CA) according to an FDA-approved manufacturer’s protocol. Location-guided computer-assisted screening of TPPT slides was accomplished using the ThinPrep Imaging System.33

HPV Test Method

Detection of hrHPV DNA in TPPT PreservCyt vial fluid was performed using the FDA-approved Hybrid Capture 2 assay method (Qiagen, Hilden, Germany),34 which tests for high-risk and intermediate-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The results of hrHPV DNA testing were either positive or negative.

Histopathologic Follow-up

At MWH, all histopathologic results of CIN2 and CIN2+ are routinely confirmed and cosigned by a second pathologist.35 Immunohistochemical stains for p16 and Ki-67 are used electively and liberally by staff pathologists to increase the reliability of a CIN2+ diagnosis.36 Histopathologic follow-up includes endocervical curettage, cervical biopsy, cervical conization using a loop electrosurgical excision procedure, or cold-knife conization. In this report, CIN terminology is used to refer solely to histopathology results, whereas HSIL and LSIL terminology is used primarily to refer to Pap test results. Time elapsed from an LSIL test result with a concurrent negative hrHPV result until...
colposcopic examination, cervical biopsy, and follow-up procedures was abstracted from record reviews extending through July 2013. Time elapsed from an LSIL test result with a concurrent negative hrHPV result until any further cytologic examination was also recorded. Any follow-up hrHPV testing, either cotesting or stand-alone HPV test requests ordered by a clinician, was recorded.

Statistical Analysis
The Pearson $\chi^2$ test was used for statistical analysis (Fisher exact test for small sample sizes) conducted on an SAS 9.1 system (SAS Institute, Cary, NC). A value of $P < .05$ was considered statistically significant.

Results

**Combined Histopathologic and Cytologic Follow-up Results**
During the study period, 680 women with HPV-negative LSIL results were identified. The mean age of these patients was 49.8 years, with a range from 20 to 89 years. Of the 680, 212 (31.2%) had no documented follow-up results in our database; 397 (58.4%) of 680 had at least one follow-up Pap test result or histopathologic follow-up result within 1 year, including 268 (39.4%) of 680 with histopathologic results and 129 (19.0%) of 680 with only cytologic follow-up results. Cases were also followed over an extended period of 100 months from June 1, 2005, through September 30, 2013. The mean extended follow-up time was 32.8 months (range, 0.5-100 months). During extended follow-up, high-grade squamous intraepithelial lesions (CIN2/3/HSIL) were documented by histopathology or cytology in 14 (3.0%) of 468 and CIN1/LSIL in 184 (39.3%) of 468.

No cases of invasive cervical carcinoma were identified during follow-up of these patients.

Follow-up Diagnoses of Histopathologic Cervical Intraepithelial Neoplasia
Among 268 patients with histopathologic follow-up within 1 year, CIN2+ was diagnosed in nine (3.4%) patients, CIN1 was diagnosed in 139 (51.9%), and 120 (44.8%) had negative/benign-reactive results. No follow-up CIN2/3 diagnoses were documented in patients 60 years or older.

Considering only histopathologic follow-up data, 303 patients had at least one histopathologic follow-up diagnosis during the extended study period. There was a mean follow-up time of 33.1 months. CIN2+ was diagnosed in four additional patients, yielding a total of 13 (4.3%) diagnoses in 303followed patients. CIN1 was diagnosed in 158 (52.1%) of 303, and 132 (43.6%) of 303 had negative/benign-reactive histopathologic follow-up results. No follow-up CIN2/3 diagnoses were documented in patients 60 years or older.

### Table 1

**Extended Study Period Age-Stratified Follow-up Outcomes After HPV-Negative LSIL**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Total No.</th>
<th>Histopathologic Follow-up</th>
<th>Cytologic Follow-up Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>CIN2/3, No. (%)</td>
<td>CIN1, No. (%)</td>
</tr>
<tr>
<td>20-29</td>
<td>58</td>
<td>34</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>30-39</td>
<td>152</td>
<td>102</td>
<td>5 (4.9)</td>
</tr>
<tr>
<td>40-49</td>
<td>131</td>
<td>95</td>
<td>3 (3.5)</td>
</tr>
<tr>
<td>50-59</td>
<td>99</td>
<td>67</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>60-69</td>
<td>22</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>≥70</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>468</td>
<td>303</td>
<td>13 (4.3)</td>
</tr>
</tbody>
</table>

CIN2/3, cervical intraepithelial neoplasia 2 or 3; CIN1, cervical intraepithelial neoplasia 1; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
Follow-up Cytologic Findings of Squamous Intraepithelial Lesions

In 129 patients with cytologic follow-up only within 1 year after HPV-negative LSIL findings, HSILs were reported in one (0.8%) of 129, LSILs were documented in 22 (17.1%) of 129, and 106 (82.2%) of 129 had negative or atypical squamous cells of undetermined significance cytology (ASCUS) results (Table 2). Examining cytologic follow-up throughout the entire longer study period, 165 patients had only cytologic follow-up results. There was a mean extended follow-up period of 37.1 months. No additional cases of HSILs were identified. LSILs were identified in 26 (15.8%) of 165, and 138 (83.6%) of 165 had negative or ASC-US results (Table 3).

Association Between Age and Histopathologic or Cytologic Follow-up

When patients were stratified into 10-year age groups and follow-up results were assessed, no significant differences in the likelihood of CIN2/3/HSIL findings were identified between age groups, although no CIN2/3/HSIL findings were identified by histopathologic or cytologic follow-up in patients older than 60 years (Tables 2 and 3). $P$ value was .202 when the highest positive rate of CIN2/3/HSIL in women aged 50 to 59 years was compared with that in women aged 60 years or older. When all women with histologic follow-up results were divided into two groups (<60 years and ≥60 years), high-grade positive rates between these two groups were not statistically different (4.5% vs 0%; $P = .255$).

HPV Test Follow-up Results

Subsequent HPV test results after initial negative HPV-negative LSIL findings were documented during follow-up; 321 (68.6%) of 468 patients had at least one follow-up HPV test. Of these 321 patients, 271 (84.4%) remained HPV negative while 50 (15.6%) tested HPV-positive at the initial HPV test follow-up. The mean interval to first follow-up HPV result was 19.1 months (range, 1-94 months). All follow-up HPV test results for these 321 patients were then examined over the extended study period; 257 (80.1%) of 321 had only negative HPV test results, 27 (8.4%) of 321 had only HPV-positive results, and 37 (11.5%) of 321 had both negative and positive HPV results. A total of 64 women had at least one positive HPV testing result over the extended follow-up period. The mean interval to the first follow-up HPV-positive result was 23.8 months (range, 1-88 months).

Discussion

Recent consensus cervical screening guidelines adopted in 2012 include revisions in the recommended management of women 30 years and older with HPV-negative LSIL results. Previously, most women with LSIL results were recommended to have immediate colposcopy. However, with the increased recommended use of Pap and HPV cotesting in women 30 years and older, it was understood that some women undergoing routine periodic screening would have HPV-negative LSIL cotest results. A low risk of CIN2/3+ associated with HPV-negative LSILs would potentially allow for conservative management. The data set primarily relied on in formulating the most recent cervical screening guidelines is that of Kaiser Permanente Northern California (KPNC). In 2003, KPNC began a program of routine cotesting for women 30 years and older using conventional smear cytology and a separately collected HC2 vial; more recently, cytologic testing using a separately collected SurePath LBC specimen was
introduced in 2009. In this data set, the comparative 5-year risks of CIN2+ (19%) and CIN3+ (6.1%) were higher for HPV-positive LSILs than for HPV-negative LSILs (CIN2+, 6.1%; CIN3+, 2%). These data on CIN2/3+ risk were the basis of the new recommendation for preferred management of HPV-negative LSILs by cotesting in 1 year rather than immediate colposcopy.

Our study population of 468 HPV-negative women with LSILs with extended follow-up is the largest routine US clinical practice study to date on patients with FDA-approved computer-imaged LBC and HPV testing from one preservative vial.

After HPV-negative LSIL results from a single PreservCyt vial in the current study, both the CIN2+ detection rate (3.0%) and CIN3+ detection rate (0.2%) during extended follow-up were lower than rates reported by Kaiser (CIN2+, 5.1%; CIN3+, 2%). CIN3, regarded by many as a more definitive end point of cancer risk, was detected in only one case in our current study, as a focal finding within a predominant background of CIN2. Kaiser’s reported experience also differs from our findings in that cases of cervical cancer were diagnosed at Kaiser after HPV-negative LSIL results from a single LBC collection vial. In contrast, Kaiser’s standard protocol for cervical screening of women 30 years and older calls for routine collection of cytology specimens first, followed by separate collection of a second HPV specimen (confirmed in personal communication from Walter Kinney, MD, KPNC, June 23, 2014). Compared with cotesting from a single specimen vial, however, available data suggest that sequential testing could introduce sampling bias and decrease HPV detection in the second sample. This is caused by more easily friable cells being preferentially removed during the first sampling, resulting in the second sampling not necessarily being representative of the pathology that may have been present during the first sampling. This also has been noted with cervical cytology testing, resulting in cytology practice recommendations that, in cases with an unsatisfactory Pap test, the subsequent resampling of the cervix should be postponed for 6 to 12 weeks so that the cervical epithelium can regenerate.

Additional large data sets addressing the crucial clinical end point of invasive cervical cancer risk after specific cervical screening
test results and history are still needed to help inform and strengthen follow-up recommendations.

We did not identify a significant difference between age groups and risk of CIN2+ lesions after HPV-negative LSILs, with the possible exception of women 60 years and older, in whom no CIN2+ lesions were detected. In our opinion, this is most likely the result of having limited numbers of patients in the oldest age groups. Most patients in our study group were between 30 and 59 years of age. The observation that 20% of patients followed after HPV-negative LSIL results later tested HPV positive has several plausible explanations. A subsequent HPV-positive test may reflect a new infection by an oncogenic HPV virus that was not the cause of the LSIL cytologic findings in the previous Pap test. On the other hand, at the time of the first HPV test, an infected woman could have had a viral load that was initially below the limit of detection limit of the HPV test method but increased by the time of the subsequent HPV test.44

More than 30% of HPV-negative LSIL cases identified in our pathology database were not included in this study because there was no recorded cytologic or histopathologic follow-up. In Kaiser’s initial report on follow-up after HPV-negative LSILs,6 45% did not have available histopathologic results. Lack of a colposcopically visible lesion, changes in health care coverage, and noncompliance were raised as possible explanations. Despite the absence of available follow-up in a sizable subset of patients, the cohort we report is the largest reflecting FDA-approved cotesting from one LBC vial.

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References


20. Bansal M, Austin RM, Zhao C. High-risk HPV DNA detected in less than 2% of over 25,000 cytology negative imaged liquid-based Pap test samples from women 30 and older. Gynecol Oncol. 2009;115:257-261.


42. Kinney W, Fetterman B, Poitras N, et al. Cervical intraepithelial neoplasia 3+ (CIN3+) is not the right endpoint for evaluating cervical screening algorithms, as it does not reflect cancer risk accurately. Paper presented at the 45th meeting of the Society of Gynecologic Oncology; March 22-25, 2014; Tampa, FL.
