Follow-up Outcomes of a Large Cohort of Low-Risk Women With Negative Imaged Liquid-Based Cytology and Negative HPV Test Results

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Key Words: ThinPrep test; Human papillomavirus; Double-negative; Cervical intraepithelial neoplasia screening

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Abstract

Recently updated cervical screening guidelines have proposed a 5-year screening interval for women aged 30 years and older with “double-negative” Papanicolaou (Pap) and high-risk human papillomavirus (hrHPV) results (DNR); however, published US follow-up data on women with DNR tested with US Food and Drug Administration (FDA)–approved HPV testing are limited to studies from Kaiser Permanente using conventional Pap smear cytology. Between July 2005 and June 2006, 4,112 patients with DNR who were screened with computer-imaged liquid-based cytology (LBC) (ThinPrep) and Hybrid Capture 2 (HC2) hrHPV tests of LBC vial fluid were identified. Cytologic or histopathologic data were available for 3,211 patients who were followed up for a mean 44 months. Among 2,960 patients aged 30 years and older with DNR, follow-up cervical abnormalities of cervical intraepithelial neoplasia (CIN) 3 or more severe (CIN3+) were documented in 5 (0.17%), including 1 endocervical adenocarcinoma. After DNR, CIN+ diagnoses were significantly more likely in women younger than 50 years than in older women. These data are consistent with previously published US and international studies that have consistently documented low rates of histopathologic CIN3+ during years of follow-up after DNR. Large-scale nationwide data are needed to further assess the level of risk of invasive cervical cancer after DNR using different available hrHPV testing methods.
and HPV cotesting in US women aged 30 years and older has remained limited, and implementation has been highly variable.6-8 The 2012 updated guidelines for the extended 5-year screening interval cite only 1 large follow-up study of conventional Papanicolaou (Pap) smear cytology and HC2 hrHPV testing conducted in the northern California facility of Kaiser Permanente.9 To the best of our knowledge, no US follow-up reports have been published on patients with DNR tested with now prevalent FDA-approved manually screened or computer-imaged liquid-based cytology (LBC) and hrHPV test methods. Accordingly, we undertook a retrospective follow-up study of patients with DNR screened with FDA-approved computer-imaged LBC and hrHPV test results at a large US academic hospital for women.

Materials and Methods

A retrospective study was designed and initiated after obtaining approval from the institutional review board at the University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA. A computer-based search was carried out on our CoPath laboratory information system (Cerner, Kansas City, MO) to retrieve cases with DNRs over a 12-month span between July 2005 and June 2006. All specimens were processed in the Magee-Womens Hospital (MWH) of the UPMC pathology laboratory and reported using current Bethesda System 2001 terminology.10 In this report, all Bethesda System result terminology refers to cytologic interpretations. ThinPrep Pap tests (Hologic, Marlborough, MA) were prepared according to the manufacturer’s specifications from PreservCyt samples using an automated processor (ThinPrep 3000). Slides were stained using a Sakura Tissue Tek automated slide stainer (Sakura Finetek USA, Torrance, CA) according to an FDA-approved manufacturer’s protocol. ThinPrep Pap test slides were evaluated with location-guided computer-assisted screening using the ThinPrep imaging system.11 The MWH cytology laboratory is a large, subspecialized academic hospital laboratory that usually reports more than 100,000 Pap tests per year from a large, integrated hospital health system that serves a metropolitan area with a significantly older population profile than the national average.12 UPMC is a large, integrated private health system in which Pap tests are collected by a highly diverse group of clinical providers that includes gynecologists, family physicians, internists, nurse practitioners, physician assistants, and house-staff trainees. The laboratory reporting profile has been documented in numerous publications.13-27

hrHPV DNA testing was ordered by clinicians according to the following ordering options: reflex testing triggered by indeterminate abnormal atypical squamous cell Pap test results, cotesting with Pap tests in women aged 30 years and older, and cotesting regardless of age or Pap test results. If hrHPV DNA was detected on negative Pap smears, the Pap test slides were routinely manually rescreened by the screening cytotechnologist, referred for further manual rescreening by a quality assurance cytotechnologist, and then also reviewed by a pathologist. hrHPV DNA detection was performed by the commercially available, FDA-approved HC2 method.

Cytologic and histopathologic follow-up results and repeat hrHPV test results for these women with DNR were recorded. Histopathologic follow-up included endocervical curettage, cervical biopsy, and cervical excisional procedures using either the loop electrosurgical excision procedure or cold knife conization. The follow-up period was defined as the time from an original negative Pap test finding and negative hrHPV test until the last cervical histopathologic or cytologic examination. For the cases with CIN2/3 diagnosed on histopathologic examination, the date of the diagnosis was counted as the end point of follow-up. In this report, all CIN terminology refers to histopathologic results. Women who had only repeat Pap tests were also included in this study. For women undergoing 2 or more procedures during the follow-up period, only the most abnormal histopathologic diagnosis was recorded.

The primary follow-up outcomes were low-grade squamous intraepithelial lesion (LSIL)/CIN1 and more serious lesions (LSIL/CIN+), including CIN2/3, vaginal intraepithelial neoplasia 2/3, high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma in situ (AIS), and invasive carcinoma. Categorical data were compared using standard contingency table analyses based on the χ² test or Fisher exact test for small numbers using the SAS 9.3 system (SAS Institute Inc, Cary, NC). P values of less than .05 were considered statistically significant.

Results

During the 12-month study interval between July 1, 2005, and June 30, 2006, a total of 4,112 women with DNR were identified. Among these women, 901 (21.9%) had no documented cytologic or HPV testing or histopathologic follow-up after at least 5 years and were excluded from this study. Cytologic (with or without HPV testing) and/or histopathologic follow-up results on 3,211 women were included in the study. The average age of these 3,211 women was 46.8 years (range, 15-87 years) at the time of the initial negative cotesting results. The average follow-up period was 44 months (range, 3 to 69 months). Histopathologic and cytologic follow-up results are shown in Table II. CIN1/LSIL and more severe lesions (CIN1/LSIL+) were documented in 86 (2.7%) of 3,211 patients. Lesions of CIN2 or higher severity (CIN2+) were documented in 12 (0.4%) of 3,211 patients,
including histopathologic diagnoses in 5 cases of CIN2/3 and 6 glandular neoplasias (1 AIS, 1 invasive cervical adenocarcinoma, and 4 endometrial malignant neoplasms) and cytologic diagnosis in 1 HSIL case (no histologic diagnosis).

When women with DNR were divided into 2 age groups (<50 and ≥50 y), the incidence of follow-up CIN1/LSIL+ diagnoses was 3.5% (65/1847) in women younger than 50 years, significantly higher than the rate of CIN1/LSIL follow-up diagnoses of 1.1% (15/1,364) in women aged 50 years or older (P < .0001). The incidence of follow-up diagnoses of glandular neoplasias was 0.05% (1/1,847) in women younger than 50 years and 0.37% (5/1,364) in women aged 50 years or older; however, this difference was not statistically significant (P = .090, Fisher test). Seven of 8 women with DNR and follow-up CIN2+ diagnoses were younger than 50 years old.

Fifty (67.6%) of 74 patients with DNR with CIN1/LSIL follow-up findings, 5 of 6 patients with CIN2/3 follow-up results, and all 6 cases with subsequent glandular neoplasia diagnoses were diagnosed on histopathologic follow-up examination (Table 2). In all 12 DNR cases with follow-up diagnoses of glandular neoplasia or CIN2/3/HSIL, the average interval between the index DNR and initial follow-up diagnosis (1 case with Pap test follow-up only) was 28.5 months (range, 2-64 mo); for CIN2/3/HSIL, 37.3 months (range, 11-64 mo); and for glandular neoplasias, 19.3 months (range, 2-48 mo). The average follow-up period from initial DNR to a subsequent Pap test was 24 months (range, 3-68 mo). The average number of subsequent Pap tests was 2.3 (range, 3-12).

During the follow-up period, 2,023 (63.0%) of 3,211 women with DNR with histopathologic or cytologic follow-up had at least 1 repeat hrHPV test. On follow-up hrHPV testing, the numbers of women with positive, negative, and both positive and negative results are detailed in Table 3. A total of 107 women (5.3%) had hrHPV-positive results during the follow-up period. An initial repeat hrHPV test was positive in 89 (4.4%) of 2,023 women (Table 4). The average follow-up interval to an initial repeat hrHPV test was 31 months (range, 3-69 mo). The average follow-up interval to an initial repeat positive hrHPV test was 34 months (range, 5-69 mo). The average number of repeat hrHPV tests was 1.34 (range, 1-6).

LSIL/CIN1+ results were documented in a significantly greater proportion of women with DNR and positive repeat hrHPV results than in DNR women with negative repeat hrHPV results (46.4% vs 1.8%; P < .001). LSIL/CIN1+ results were also documented in a significantly greater proportion of women with DNR and both positive and negative follow-up hrHPV results than in women with DNR and only negative hrHPV follow-up testing (29.4% vs 1.8%; P < .001). LSIL/CIN1+ results were also identified in a significantly greater proportion of patients with DNR and only positive follow-up hrHPV results than in patients with both positive and negative hrHPV follow-up test results (46.4% vs 29.4%; P < .001). Women with DNR diagnosed during follow-up with endometrial malignancies had negative hrHPV follow-up test results.

Table 1
Follow-up Findings in 3,211 Patients With “Double-Negative” HPV and LBC Results

<table>
<thead>
<tr>
<th>Age, y</th>
<th>No. of Patients</th>
<th>No. of Patients with Follow-up</th>
<th>Glandular Neoplasia</th>
<th>CIN2/3/HSIL*</th>
<th>CIN1/LSIL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>48</td>
<td>38</td>
<td>0</td>
<td>0</td>
<td>4 (10.5)</td>
</tr>
<tr>
<td>20-29</td>
<td>285</td>
<td>213</td>
<td>0</td>
<td>2 (0.9)</td>
<td>18 (8.5)</td>
</tr>
<tr>
<td>30-39</td>
<td>830</td>
<td>667</td>
<td>1</td>
<td>1 (0.1)</td>
<td>25 (3.8)</td>
</tr>
<tr>
<td>40-49</td>
<td>1,172</td>
<td>929</td>
<td>0</td>
<td>3 (0.3)</td>
<td>12 (1.3)</td>
</tr>
<tr>
<td>50-59</td>
<td>1,154</td>
<td>915</td>
<td>2</td>
<td>0</td>
<td>12 (1.3)</td>
</tr>
<tr>
<td>≥60</td>
<td>623</td>
<td>449</td>
<td>3</td>
<td>0</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Total</td>
<td>4,112</td>
<td>3,211</td>
<td>6 (0.2)</td>
<td>6 (0.2)</td>
<td>74 (2.3)</td>
</tr>
</tbody>
</table>

CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus; LBC, liquid-based cytology; LSIL, low-grade squamous intraepithelial lesion.

* Data are given as number (percentage).

Table 2
Follow-up Findings in 3,211 Patients With “Double-Negative” HPV and LBC Results and Available Histopathologic or Cytologic Follow-up Results

<table>
<thead>
<tr>
<th>Follow-up Method(s)</th>
<th>Total No. of Cases</th>
<th>Glandular Neoplasia*</th>
<th>CIN2/3/HSIL</th>
<th>CIN1/LSIL</th>
<th>CIN/SIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology</td>
<td>549</td>
<td>6</td>
<td>5 (0.9)</td>
<td>50 (9.1)</td>
<td>55 (10.0)</td>
</tr>
<tr>
<td>Cytology only</td>
<td>2,662</td>
<td>0</td>
<td>1 (0.04)</td>
<td>24 (0.9)</td>
<td>25 (0.9)</td>
</tr>
<tr>
<td>Total</td>
<td>3,211</td>
<td>6 (0.2)</td>
<td>6 (0.2)</td>
<td>74 (2.3)</td>
<td>80 (2.5)</td>
</tr>
</tbody>
</table>

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LBC, liquid-based cytology; LSIL, low-grade squamous intraepithelial lesion.

* Includes diagnoses of 1 adenocarcinoma in situ, 1 invasive cervical adenocarcinoma, and 4 endometrial carcinomas. Data are given as number (percentage) unless otherwise indicated.
Detailed information on 12 patients with DNR with more severe cervical abnormalities (CIN2/3/AIS+) or endometrial malignancy documented on follow-up is summarized in Table 5. The average age of the 8 patients with DNR and later diagnoses of high-grade CIN+ was 38.8 years, and the 4 patients with follow-up diagnoses of endometrial malignancy was 61.5 years. Four of 8 patients with DNR and later diagnoses of high-grade CIN underwent repeat HPV testing during the follow-up period, and all were positive. Among 8 patients with DNR and later high-grade CIN+, 4 had previous diagnoses of CIN and/or a glandular abnormality (atypical glandular cells/AIS) within 3 years. No abnormal history was noted in 4 patients with follow-up diagnoses of endometrial malignancy. Of 4 patients with DNR with endometrial malignancy, 2 underwent repeat HPV tests, and both had negative results.

**Discussion**

During an average follow-up period of 44 months, 12 (0.4%) of 3,211 patients with DNR and available cytologic or histopathologic follow-up had documented CIN2+ diagnoses on follow-up (Table 2); this included 6 CIN2/3/HSIL, 1 AIS, 1 endocervical adenocarcinoma, and 4 endometrial malignancies (Table 5). Excluding diagnoses of endometrial neoplasms, cervical CIN2/AIS+ follow-up diagnoses were documented in 8 (0.25%) of 3,211 patients who were followed up after baseline DNR. Among 2,960 patients with DNR who were 30 years of age and older, cervical CIN2/AIS+ follow-up diagnoses were documented in 6 patients (0.2%) and CIN3/AIS+ diagnoses in 5 (0.17%). Cervical CIN+ diagnoses after DNR were significantly more likely in women younger than 50 years than in women aged 50 years and older.

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**Table 4**

First Follow-up HPV Test Result after “Double-Negative” HPV and LBC Results

<table>
<thead>
<tr>
<th>HPV Follow-up Results</th>
<th>No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>First follow-up test HPV-positive</td>
<td>89 (4.4)</td>
</tr>
<tr>
<td>First follow-up test HPV-negative</td>
<td>1,934 (95.6)</td>
</tr>
<tr>
<td>Total</td>
<td>2,023 (100)</td>
</tr>
</tbody>
</table>

HPV, human papillomavirus; LBC, liquid-based cytology.

**Table 5**

CIN2/3/HSIL/AIS+ Diagnoses After “Double-Negative” HPV and LBC

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>Diagnosis</th>
<th>Interval, mo</th>
<th>Repeat HPV Test</th>
<th>Diagnosis</th>
<th>Interval, mo</th>
<th>History of Biopsy or LBC Prior 3 y</th>
<th>History of HPV Testing Prior 3 y</th>
<th>Follow-up Result</th>
<th>Interval, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>CIN3</td>
<td>64</td>
<td>Pos</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>CIN3</td>
<td>11</td>
<td></td>
<td>CIN2</td>
<td>4</td>
<td>CIN1</td>
<td>Pos</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>CIN3</td>
<td>11</td>
<td>Pos</td>
<td>1 Neg LBC</td>
<td>18</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>CIN2</td>
<td>51</td>
<td>Pos</td>
<td>3 Neg LBC</td>
<td>2</td>
<td>AIS/CIN2</td>
<td>AGC</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>CIN2</td>
<td>49</td>
<td>Pos</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>HSIL</td>
<td>38</td>
<td></td>
<td>AIS</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>AIS</td>
<td>12</td>
<td>Neg</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>Cervix- AdCa</td>
<td>24</td>
<td>Neg</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>EM-CaSarc</td>
<td>24</td>
<td>Neg</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>EM-AdCa</td>
<td>24</td>
<td>Neg</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>57</td>
<td>EM-CaSarc</td>
<td>6</td>
<td>Neg</td>
<td>3 Neg LBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>55</td>
<td>EM-CaSarc</td>
<td>48</td>
<td>Neg</td>
<td>3 Neg LBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AdCa, adenocarcinoma; AGC, atypical glandular cells; AIS, adenocarcinoma in situ; CaSarc, carcinosarcoma; EM, endometrial; hrHPV, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; LBC, liquid-based cytology; NA, not available; Neg, negative; Pos, positive.
By comparison, in a pooled study of approximately 25,000 women enrolled in 7 European cohorts, the cumulative incidence rate of CIN3+ after a negative HPV test result at baseline was 0.3% over 6 years of follow-up. Similarly, in a retrospective observational California study conducted by Kaiser of 330,000 women aged 30 years and older who were cotested at 3-year intervals, the 3-year risk of CIN3+ after a negative HPV test result alone was reported to be 0.17%. Therefore, our data are consistent with previously published studies that have consistently documented low rates of histopathologic CIN3+ during years of follow-up after DNR, including studies performed using various cell sampling and hrHPV testing methods.

Risk for both cervical CIN2+ and LSIL/CIN1+ after DNR were greater in women younger than 50 years than in those aged 50 years and older. These observations are consistent with other studies which have shown that the risk of significant cervical disease after negative HPV test results decreases as women age and become less likely to acquire new infections; even when such infections are acquired, they tend to resolve as in younger women.

All 4 patients with DNR, follow-up HPV testing, and later CIN2/3 diagnoses had positive follow-up HPV test results, suggesting that cotesting was valuable in follow-up. Among 2,023 patients with DNR and at least 1 follow-up HPV test, our data also shows that LSIL/CIN1 results were significantly more likely in women with at least 1 positive HPV test result than in women with only negative follow-up HPV test results. These results point to the negative predictive value of HPV testing as well as the limited specificity of hrHPV testing in predicting more significant CIN2+ lesions.

The most important goal of cervical screening is to increase the “cure proportion” of screened women, which includes both prevention of invasive cervical cancers by detection and ablation of precancers and also screening-induced downstaging of prevalent cancers to lower and more favorably treatable stages. Unfortunately, very large cohorts are needed to assess invasive cancer risks for each possible cytopathologic abnormality and HPV test result, including DNR. Our study population was too small and the follow-up period too short to precisely document the effect of DNR in our laboratory on subsequent invasive cervical cancer risk. One 66-year-old patient was diagnosed with endocervical adenocarcinoma 12 months after DNR. Twenty-six months before DNR and 38 months before she received a histopathologic diagnosis of cervical adenocarcinoma, this patient had LBC findings of atypical glandular cells, with benign endocervical and endometrial findings on follow-up. This case highlights some of the well-known challenges of sampling and diagnosing endocervical adenocarcinoma, even after abnormal screening results. Some reports have suggested that older women with endocervical cancer are more likely to test HPV negative.
widely used, along with recommended extended screening intervals, a targeted data collection effort is needed.

Finally, it should be acknowledged that 4 cases of endometrial malignancy (2 adenocarcinomas and 2 carcinomas) were diagnosed on follow-up of women with baseline DNR. Although a significant number of endometrial carcinomas may be cytologically detectible and possibly enhanced by LBC methods, the clinical benefit of such detection, in terms of increased survival, remains unproven.47

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