Clinical Significance of Cytologic Diagnosis of Atypical Squamous Cells, Cannot Exclude High Grade, in Perimenopausal and Postmenopausal Women

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Abstract

We used cytohistologic correlation to determine the clinical significance of atypical squamous cells, cannot exclude high grade (ASC-H), in perimenopausal and postmenopausal women. A computer search identified 250 Papanicolaou smears from women older than 45 years with a diagnosis of ASC-H. Cases were considered perimenopausal (45 to <55 years; 150 cases) and postmenopausal (≥55 years; 100 cases). No follow-up data were available for 33 cases, which were excluded. The remaining 217 cases (perimenopausal, 127; postmenopausal, 90) had surgical or cytologic follow-up. Results of follow-up colposcopic biopsy were available for 176 (81.1%) and cytology for 41 (18.9%) women. Follow-up results were as follows: perimenopausal women, negative, 50 (39.4%); mild dysplasia (low-grade squamous intraepithelial lesion [LSIL]), 46 (36.2%); high-grade dysplasia (high-grade SIL [HSIL]); 28 (22.0%); and ASC of undetermined significance (ASC-US), 3 (2.4%); postmenopausal women, negative, 52 (58%); LSIL, 31 (34%); HSIL, 5 (6%); and ASC-US, 2 (2%). The diagnosis of ASC-H in perimenopausal women usually is associated with LSIL or a negative diagnosis on follow-up, suggesting a less aggressive surveillance and treatment regimen is needed for postmenopausal women with ASC-H.

The Bethesda System (TBS) was established to facilitate communication between cytopathologists and clinicians for appropriate management of patients. The recognition that high-grade dysplasia (high-grade squamous intraepithelial lesion [HSIL]) is likely to progress to invasive cancer, whereas most low-grade lesions regress spontaneously, raises awareness that eradicating HSIL is critical for cancer prevention.1,2 This knowledge has prompted efforts to improve early detection of HSIL.

Although most women with atypical squamous cells do not require immediate colposcopic examination or aggressive management, 5% to 10% of them might harbor an underlying HSIL, which requires immediate therapy.3 TBS (2001) classifies atypical squamous cells into 2 subcategories: atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells, cannot exclude high grade (ASC-H).4 This classification became necessary to facilitate cost-effective clinical management.5 Previous studies have reported that ASC-H is associated with a significant incidence of HSIL on follow-up compared with ASC-US.6-11 However, the rate of concurrent and subsequent HSIL in biopsy specimens from patients with ASC-H in the literature ranged from 29% to 75%.6-11

Owing to demographics in the United States and the nature of cervical cancer, the majority of women participating in Papanicolaou (Pap) smear screening are younger than 50 years.12 It is estimated that by the next decade, postmenopausal women will constitute about one fifth of the adult population.13 Therefore, the number of postmenopausal patients subjected to Pap smears most likely will increase because the majority of these women will be familiar with having annual Pap smears.12
A number of investigators have studied the diagnosis of squamous atypia in postmenopausal women and have had conflicting results.14-17 Most studies demonstrated that squamous atypia in postmenopausal women rarely is associated with biopsy-proven dysplasia or human papillomavirus (HPV) DNA detection.14,15 Certain specific problems are associated with the assessment of specimens from postmenopausal women, which can be diagnostically challenging.13,18,19 Artifactual alterations, ranging from drying and inflammatory changes, reactive metaplasia, presence of naked nuclei, and pseudoparakeratosis to sampling problems, are common in cervicovaginal smears from postmenopausal women.18 These alterations may lead to the overdiagnosis of squamous atypia. In addition, estrogen deficiency may lead to cytolgic changes that mimic high-grade disease.19 The significance of ASC-H diagnoses in postmenopausal women remains unclear and not fully studied. With the increase in older women in the population, it is important to determine the clinical implications of ASC-H in postmenopausal women.

The purpose of the present study was to evaluate the clinical significance of reporting ASC-H on cervical samples processed by the ThinPrep (Cytyc, Boxborough, MA) technique in postmenopausal women in comparison with perimenopausal women.

Materials and Methods

The study was approved by the University of Pittsburgh (as part of the ASC-H study) and Allegheny General Hospital Institutional Review Board (Pittsburgh, PA). A computer search of the archives of both hospitals identified all gynecologic specimens diagnosed as ASC-H during the period January 2003 through June 2004. The search retrieved 800 cases of ASC-H of 152,495 Pap smears for 18 months, including 250 cases in women older than 45 years. Women were divided arbitrarily into perimenopausal (45 to <55 years) and postmenopausal (≥55 years) categories.

ThinPrep Processing

All specimens were obtained using the ThinPrep technique consisting of a broom-type sampling device (Papette, Wallach Surgical Devices, Gaithersburg, MD) or a combination plastic spatula and Cytobrush (Cytyc). The cellular sample was collected and rinsed into a vial of PreservCyt Solution (Cytyc). The solution was processed with the ThinPrep 2000 automated slide processor (Cytyc).

Thin-layer slides were prepared using the ThinPrep 2000 automated slide processor according to the manufacturer’s instructions. Briefly, the vial containing the cells is placed in the processor, and a dispersion cycle homogenizes the cell suspension. The cells are automatically collected on a polycarbonate filter membrane. A thin, evenly dispersed monolayer of cells is deposited from the filter onto the slide in a 20-mm circle. Extraneous mucus and blood are removed in the process. The slides are removed manually from the processor and stained by the Pap method.

Reporting System

Cytologic diagnoses and specimen adequacy were classified by using TBS (2001) for cervical cytology.3 The new diagnostic category ASC-H reflects a mixture of true HSIL and its mimics. An interpretation of ASC-H was made when the atypical squamous cells resembled single squamous metaphastic cells. The cells might be euchromatic or slightly hyperchromatic with variable nuclear enlargement, nuclear membrane irregularity, and increased nuclear/cytoplasmic (N/C) ratios. ASC-H also is diagnosed when atypical cells are arranged in loosely cohesive groups or in syncytial fragments. The slides were reviewed by 2 cytopathologists (R.S.S. and A.K.-S.) to confirm the diagnosis of ASC-H and to analyze the following cytomorphologic parameters: inflammatory background, arrangement (single cells and clusters), chromatin pattern, and nuclear membrane irregularity (including grooves). These features were compared between groups with SIL follow-up and benign follow-up.

Follow-up and Histologic Data

The method and results of patient evaluation, which included repeated Pap tests, colposcopic examination along with endocervical curettage and cervical biopsy, loop electrosurgical excision procedure, and hysterectomy, were reviewed and recorded in Table 1. Follow-up Pap tests and all biopsies and curettages were performed within 6 months. All biopsy specimens were formalin fixed, paraffin embedded, and stained with H&E. Three-level sections were reviewed without knowledge of patient identification, previous diagnosis, cytologic interpretation, and follow-up data. For the purposes of this study, the histologic diagnoses of HPV and cervical intraepithelial neoplasia (CIN) 1 are classified as low-grade SIL (LSIL) and CIN 2 and CIN 3 as HSIL.

High-risk-type HPV testing was performed on the residual samples after slide preparation, using Digene Hybrid Capture II (Digene, Gaithersburg, MD). The results of HPV testing were reported as positive or negative.

Results

During the study period, the total number of Pap tests accessioned and examined was 152,495 Pap smears. A total of 800 cases were identified with an interpretation of ASC-H, accounting for 0.52% of all Pap tests screened during the study period. During the same period, 7.3% of the specimens...
were classified as atypical squamous cells (ASC). As a result, ASC-H accounted for 7.1% of specimens with an ASC interpretation. The mean ± SD age of perimenopausal women with an ASC-H interpretation was 49 ± 3 years (range, 45 to <55 years), whereas in postmenopausal women, the mean ± SD was 61 ± 7 years (range, 55-78 years).

Of the 250 cases with an interpretation of ASC-H in women older than 45 years, 33 were excluded owing to lack of follow-up. Of the remaining 217 cases, 176 (81.1%) had histologic follow-up, and 41 (18.9%) had repeated Pap test results within 6 months. Cases were divided into 2 groups: perimenopausal, 127 cases; and postmenopausal, 90 cases.

### Perimenopausal Women

Follow-up was available in 127 cases. Follow-up colposcopy and cervical biopsy were performed in 105 cases (82.7%) and cytologic follow-up in 22 (17.3%; Table 1). Cervical biopsy follow-up findings were as follows: "negative for intraepithelial lesions or malignancy," 33; LSIL (CIN 1), 44; and HSIL (CIN 2 or CIN 3), 28. Pap test follow-up findings were as follows: "negative for intraepithelial lesions or malignancy," 33; LSIL, 44; and ASC-US, 28. The average number of follow-up Pap smears was 2.3 (range, 2-4). Therefore, the overall follow-up results in 127 cases were as follows: negative, 50 (39.4%); mild dysplasia, 46 (36.2%); high-grade dysplasia, 28 (22.0%); and ASC-US, 3 (2.4%) [Table 2].

### Postmenopausal Women

Follow-up colposcopy and cervical biopsy were performed in 71 (79%) of 90 cases and cytology follow-up in 19 cases (21%) (Table 1). Cervical biopsy follow-up findings were as follows: negative for intraepithelial lesions or malignancy, 37; low-grade dysplasia (CIN 1), 30; and high-grade dysplasia (CIN 2 or CIN 3), 4 [Image 1], [Image 2], and [Image 3]. Pap test follow-up findings were as follows: negative for intraepithelial lesions or malignancy, 15; LSIL, 1; HSIL, 1; and ASC-US, 2. The average number of follow-up Pap smears was 1.6 (range, 1-4). Therefore, the overall follow-up results were as follows: negative, 52 (58%); mild dysplasia, 46 (36.2%); high-grade dysplasia, 5 (6%); and ASC-US, 2 (2%) (Table 2).

None of the examined cytomorphologic features showed significant differences between the SIL and benign follow-up groups. Inflammatory background was seen more often in the benign than in the SIL follow-up group, but without a significant difference (39% vs 22%).

Approximately 83% of perimenopausal women and 79% of postmenopausal women had histologic follow-up, not a
significant difference. The positive predictive value for a diagnosis of ASC-H in perimenopausal women was 22% vs 6% in postmenopausal women. There was a significant difference between the incidence of high-grade dysplasia in perimenopausal and postmenopausal women ($P < .01$) and no significant difference (36.2% vs 34%) for low-grade dysplasia.

**Table 3** compares the high-risk HPV status with follow-up findings. The HPV test was performed in 39 (30.7%) of 127 and 25 (28%) of 90 cases, with positive results of 38% (15/39) and 36% (9/25), in perimenopausal and postmenopausal women, respectively. Patients with follow-up–proven HSIL had positive results in high-risk HPV testing, with a sensitivity and a negative predictive value for HSIL of 100%.

**Discussion**

The interpretation of cervical-vaginal smears from postmenopausal patients can be challenging. Extensive inflammation, parabasal cells with organophilic cytoplasm, nuclear variations
secondary to drying, and degeneration associated with atrophic vaginitis may result in cellular changes falsely interpreted as squamous atypia and/or a more severe lesion. Immature metaplastic and reparative changes are another cause of false-positive results, in addition to other age-related epithelial disturbances, which include prominent perinuclear halos, nuclear hyperchromasia, variation in nuclear size, and multinucleation.

TBS (2001) proposed dividing ASC into 2 groups: ASC-US and ASC-H. ASC-H is a less common interpretation. In our study, ASC-H was reported in 0.52% of all Pap tests screened during the study period and represented 7.1% of specimens with an ASC interpretation. Previous reports showed that the incidence of ASC-H ranged from 0.13% to 0.2% of Pap smears and from 6% to 9% of all specimens classified as ASC. Similar to our study, others reported a higher percentage for ASC-H in their population. This difference may be attributed to interobserver variability between laboratories or even pathologists.

Previous studies have demonstrated that patients with smears classified as ASC-H are at higher risk for HSIL than are women with ASC-US. The incidence of a clinically significant lesion following an interpretation of ASC-H was somewhere between that of ASC-US and HSIL and ranged from 29% to 79%, depending on the study. The lack of well-defined criteria for ASC-H is reflected in its poor interobserver agreement, explaining the wide difference in the literature concerning the incidence of high-grade dysplasia in follow-up biopsy specimens.

The ASC-H category includes 2 cytologic patterns. The first is atypical immature squamous metaplastic cells with a high N/C ratio and/or tissue fragments/disorganized groups of hyperchromatic cells. Most authors agree that ASC classified as ASC-H resembles metaplastic squamous cells with increased N/C ratios, mild irregular nuclear membrane, and mild hyperchromasia. In contrast with ASC-US, nuclear size is not helpful in defining ASC-H because of the wide variation in nuclear size. In our experience, the cells of ASC-H were more likely to present as isolated single cells and appear more euchromatic in liquid-based preparations. It is difficult to distinguish the morphologic features of atypical squamous metaplasia from HSIL. Atypical squamous metaplastic cells on Pap smears are known pitfalls.

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**Table 3**

<table>
<thead>
<tr>
<th>HPV Testing†</th>
<th>Positive Results‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Perimenopausal women</strong></td>
<td></td>
</tr>
<tr>
<td>Negative group (n = 50)</td>
<td>20</td>
</tr>
<tr>
<td>Low-grade group (n = 46)</td>
<td>11</td>
</tr>
<tr>
<td>ASC-US (n = 3)</td>
<td>1</td>
</tr>
<tr>
<td>High-grade group (n = 28)</td>
<td>7</td>
</tr>
<tr>
<td>Total (n = 127)</td>
<td>39</td>
</tr>
<tr>
<td><strong>Postmenopausal women</strong></td>
<td></td>
</tr>
<tr>
<td>Negative group (n = 52)</td>
<td>13</td>
</tr>
<tr>
<td>Low-grade group (n = 31)</td>
<td>7</td>
</tr>
<tr>
<td>ASC-US (n = 2)</td>
<td>0</td>
</tr>
<tr>
<td>High-grade group (n = 5)</td>
<td>5</td>
</tr>
<tr>
<td>Total (n = 90)</td>
<td>25</td>
</tr>
</tbody>
</table>

ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus.

† Number of Papanicolaou smears done with high-risk HPV test.
‡ Data are given as number (percentage) based on the number tested for HPV.
The other major cytologic pattern associated with ASC-H smears is thick tissue fragments that raise the differential diagnosis of ASC-H vs HSIL. Boon et al. proposed that these fragments might represent an artifact resulting from forceful scraping with the endocervical brush. In postmenopausal women, these tissue fragments are seen frequently. The presence of cellular overlapping, loss of polarity, and nuclear aberrations might lead to misinterpreting these smears as abnormal. The absence of isolated, definitively identifiable HSIL cells in these cases is helpful, but their absence does not exclude HSIL. Despite the recent description of these cytologic patterns, the subjectivity involved in interpreting these smears remains problematic. However, Saad et al. and Abati et al. showed that nuclear hyperchromasia and irregular nuclear contours in single cells with high N/C ratios are most helpful for accurately diagnosing SIL in postmenopausal smears diagnosed as HSIL. Our study showed that none of the cytomorphic features showed significant differences between the negative and SIL groups in follow-up of patients with ASC-H smears.

Studies consistently have demonstrated a lower detection rate of dysplasia in follow-up cervical biopsies after a cytologic diagnosis of ASC-US in older postmenopausal women. Two studies reported that the majority of older women with abnormal cytologic findings had negative follow-up evaluation results. This may be explained partly by the fact that atrophic cervical-vaginal smears exhibit uniform nuclear enlargement in the squamous cell population. Pathologists aware of age-related changes were able to make appropriate adjustments and to avoid overcalling ASC-US in smears from postmenopausal women and in Pap smears with obvious or subtle atrophy.

There have been few previous studies of the clinical significance of atypical metaplastic cells or ASC-H in postmenopausal women; results have been contradictory, and various age cutoffs were used. Some studies reported no difference in the SIL detection rate in patients younger or older than 40 years after interpretation of atypical squamous metaplastic cells (equal to ASC-H). The finding of other studies are in agreement with our results—patients who had SIL histologic diagnoses were more often younger than patients who had benign histologic diagnoses after a cytologic interpretation of atypical squamous metaplastic cells (ASC-H). Therefore, their experience and ours suggest that older women with an interpretation of ASC-H are less likely to have HSIL than are younger women.

Estrogen deficiency and tamoxifen may lead to cytologic changes that mimic high-grade disease and may induce histologic changes, including "pseudokoiolcytosis." Yang et al. and Opjorden et al. reported the presence of "small cells" of uncertain origin in the Pap smears of patients receiving tamoxifen. The incidence of small cells increases with age, and morphologically they are similar to parabasal or reserve cells normally observed in physiologically atrophic smears of postmenopausal women. We speculate that these small cells also might be confused with ASC-H, particularly in a Pap test with complete maturation background. In our study, 7 postmenopausal women were receiving tamoxifen.

The high-risk-type HPV test has been studied as an adjunctive test for the evaluation of ASC-H. In the present study, the HPV test was performed in 30.7% and 28% with positive results in 38% and 36% in perimenopausal and postmenopausal women, respectively. Similarly, Rowe et al. reported that HPV positivity was 37.5% in ASC-H. Other investigators have reported a higher HPV positive rate, up to 86% in ASC-H. This variation of HPV results may reflect the difficulty in interpretation of ASC-H, demonstrated by low interobserver agreement for ASC-H. The high-risk HPV test showed excellent sensitivity and negative predictive value for underlying HSIL (100%) but with a low positive predictive value. Saad et al. and Srodon et al. suggested that HPV testing may be helpful for selecting patients with ASC-H who should undergo colposcopic examination.

In 2002, the American Society of Colposcopy and Pathology (ASCCP) published updated guidelines for the management of patients with abnormal Pap test results. Patients with a cytologic interpretation of ASC-US should undergo reflex HPV DNA testing or repeated Pap testing, whereas patients with a cytologic interpretation of ASC-H should undergo colposcopic examination and cervical biopsy. Some studies suggested that age could be considered in the initiation or choice of management of abnormal Pap smears. However, ASCCP management guidelines did not stratify follow-up strategies for any of TBS results based on age. Our finding of HSIL on follow-up examination after the diagnosis of ASC-H in postmenopausal women was less than 6%. A recent study also reported that few prevalent cancers would be missed by using a strategy of serial cytology for asymptomatic older women.

ASC-H in postmenopausal women was associated with HSIL in fewer than 6% of cases in our population as opposed to 22% in perimenopausal women. Therefore, we suggest Pap smear follow-up with HPV testing for postmenopausal women diagnosed with ASC-H by Pap test, although colposcopy follow-up may be appropriate when compliance is an issue.

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