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Case 32-2003: A 37-Year-Old Woman with Atypical Squamous Cells on a Papanicolaou Smear
Annekathryn Goodman, M.D., and David C. Wilbur, M.D.

Presentation of Case

A 37-year-old woman was referred to the colposcopy clinic because of two Papanicolaou smears showing atypical squamous cells of undetermined significance (ASC-US).

One and two years previously, the patient had had Papanicolaou smears that were reported to be normal. Six months before referral, a routine pelvic examination revealed no abnormalities. A Papanicolaou smear at that time was interpreted as revealing ASC-US. The patient was reexamined three months later, and another Papanicolaou smear was again interpreted as showing ASC-US.

The patient (gravida 5, para 4) had had one spontaneous first-trimester abortion. She was a native of El Salvador but had resided in the United States for several years. She reported having a long-standing monogamous sexual relationship. The result of a skin test for tuberculosis with purified protein derivative, performed in the past, was positive; a chest radiograph at that time was normal. She received isoniazid for one year. Two years earlier, during her most recent pregnancy, tests for human immunodeficiency virus infection, syphilis, gonorrhea, and chlamydia had all been negative.

A general physical examination revealed no abnormalities. On pelvic examination, there were no vulvar, vaginal, or cervical lesions. Colposcopic examination revealed a cervical ectropion involving the greater part of the exocervix. The transformation zone was examined visually in its entirety and appeared normal. A repeated Papanicolaou test was performed and reportedly showed no abnormalities. Endocervical curettage was performed, and pathological examination of the specimen disclosed normal endocervical epithelium.

A diagnostic test was performed.

Differential Diagnosis

Dr. Annekathryn Goodman: This patient had two abnormal Papanicolaou smears, and in both the abnormality of the squamous cells was described as being of unknown significance. The appropriate management in such cases has been an area of controversy, but recent developments may simplify the approach.
Cervical cancer is one of the leading causes of cancer-related death in women. However, there is epidemiologic evidence that the rates of cervical cancer have fallen in all areas where screening programs have been instituted. The Papanicolaou ("Pap") test, a cytologic evaluation of exfoliated epithelial cells from the lower genital tract, is the gold standard for cervical cancer screening. The Papanicolaou test is effective because cervical neoplasia has a long preinvasive phase before the development of invasive disease; cytologic examination can detect these preinvasive lesions, which can then be eradicated.

This patient’s Papanicolaou smears were interpreted as showing ASC-US, indicating that the cytopathologist saw an abnormality in the squamous cells that did not fulfill the criteria for dysplasia but that were not readily categorized as reactive changes. ASC-US may be the finding in as many as 10 percent of all Papanicolaou smears. The majority of women with ASC-US do not have a clinically significant lesion; the abnormalities in the cells are instead due to artifact, inflammation, or estrogen deficiency. However, about 20 percent of women with ASC-US on a Papanicolaou smear are subsequently found to have a dysplastic lesion on cervical biopsy (Table 1). Thus, the ASC-US finding presents practitioners with a challenge in determining how to proceed with further evaluation.

In a patient with suspected cervical squamous-cell dysplasia, the next step after the Papanicolaou test is colposcopic examination of the cervix and biopsy of suspicious areas in order to confirm or rule out a diagnosis of dysplasia and, if dysplasia is present, to determine its grade. The decision to refer a woman such as the patient under discussion for cervical cancer screening.

**Table 1. Histologic Findings on Examination of Biopsy Specimens from Women Whose Papanicolaou Smears Showed Atypical Squamous Cells of Undetermined Significance.**

| Histologic Finding                          | No. of Patients (%) |
|--------------------------------------------|---------------------|
| Normal                                     | 783 (80.5)          |
| Cervical intraepithelial neoplasia          |                     |
| Grade 1                                    | 125 (12.8)          |
| Grade 2                                    | 64 (6.6)            |
| Invasive cancer                            | 1 (0.1)             |
| Total                                      | 973 (100.0)         |

* The data are from Manos et al.
PATHOLOGICAL DISCUSSION

Dr. David C. Wilbur: Some of the squamous cells in this patient’s cervical cytologic smear showed alterations in the nuclei consisting of enlargement, slight hyperchromasia, and irregularities of the nuclear envelope (Fig. 1). For this reason, the interpretation was “atypical squamous cells” of the “undetermined significance” subtype (so-called ASC-US).

As Dr. Goodman has said, a specimen for which the cytologic results are equivocal is described as having atypical squamous cells: there are cytologic abnormalities, but they are not sufficient for an outright diagnosis of either low-grade or high-grade pathological discussion

Figure 1. Cervical Specimen Showing Features of Atypical Squamous Cells of Undetermined Significance (Papanicolaou Stain, ×600).

Panel A shows an individual squamous cell with an enlarged nucleus (arrow). Panel B shows a squamous cell with an enlarged nucleus with hyperchromasia and mild irregularity of the nuclear envelope (arrow). Panel C shows a plaque of hyperkeratotic cells indicative of abnormal hypermaturation of the squamous epithelium. Panel D shows a group of reactive cells with infiltration by inflammatory cells and prominent nucleoli. Panel E shows a group of cells with evidence of hypermaturation with keratohyaline granule formation (arrowhead) and perinuclear clearing (arrow), which is suggestive but not diagnostic of human papillomavirus infection. Taken together, these features suggest but do not confirm the presence of a low-grade squamous intraepithelial lesion, but the suggestion of that lesion warrants additional studies; hence, the interpretation of ASC-US was rendered.
High-grade squamous epithelial lesion

Atypical squamous cells

Cytologic Interpretation (Papanicolaou smear)

| Classification | Pathological Interpretation (Biopsy) |
|---------------|-------------------------------------|
| Atypical squamous cells | Variable |
| Undetermined significance (ASC-US) | |
| Possible high-grade dysplasia (ASC-H) | Cervical intraepithelial neoplasia, grade 1 |
| Low-grade squamous epithelial lesion | Cervical intraepithelial neoplasia, grade 1 |
| High-grade squamous epithelial lesion | Cervical intraepithelial neoplasia, grade 2 or 3 |

* The information is from Solomon et al.5

Some patients with atypical squamous cells have dysplastic lesions, whereas some have non-neoplastic conditions with morphologic features on the Papanicolaou smear that overlap with those of dysplastic lesions. The changes in the squamous cells may be quite varied, and interobserver and intraobserver reproducibility in the interpretation of these specimens is poor. Conditions that mimic dysplasias include reactive cellular changes due to tissue damage caused by infection, trauma, or radiation; physiological changes secondary to hormone use and menopausal status; and a wide variety of artifacts resulting from the preparation of the specimen. Fortunately, the latter are minimized by new, liquid-based preparation methods that virtually eliminate air-drying, smearing, and fixation effects. The clinically important entities that may be characterized by atypical squamous cells are true dysplasias and even, in rare instances, invasive carcinomas. The problem, as in this case, is that morphologic features alone do not allow us to predict the risk of such lesions in an individual patient.

Findings of atypical squamous cells typically constitute between 3 and 5 percent of a cytology laboratory’s cases. However, indiscriminate use of this designation may increase the percentage to as high as 10 to 15 percent in some laboratories. Dysplasia is found on follow-up biopsy in anywhere from 10 percent to as many as 50 percent of the cases, an indication of the wide range of interpretive differences among cytologists. High-grade lesions (cervical intraepithelial neoplasia, grade 2 or 3) typically constitute 20 to 30 percent of the total. Therefore, for quality-assurance purposes, it is important for each laboratory to monitor the ratio of findings of atypical squamous cells to dysplasia, which should be in the range of 2:1 to 3:1.

Recently, testing for HPV infection has emerged as an important tool in the management of cases in which atypical cells have been found on the Papanicolaou smear. HPV infection is associated with virtually all invasive carcinomas of the cervix, both squamous and glandular, as well as with their precursor dysplastic lesions. Therefore, the presence or absence of HPV should be useful in deciding which cases of atypical squamous cells require triage to colposcopy and which do not.

HPV can be broadly classified into two groups, one associated with cutaneous infections such as the common wart and the other associated with infections and neoplasms of the genital tract. The latter group is further subdivided into low-risk and high-risk types. The former generally cause external genital warts and condyloma, whereas the latter are predominantly (though not exclusively) responsible for cervical neoplasia. Of the high-risk types, the most common are HPV types 16, 18, 31, 33, 35, and 45, which together account for about 85 percent of those detected in cervical-cancer specimens. A variety of other, less prevalent types are responsible for disease in the remaining small percentage of cases. There are geographic variations in the minor, less prevalent viral types associated with cancer; however, in all locations studied, HPV type 16 (HPV-16) and the types related to it predominate.

High-risk HPV infection of the cervical epithelium occurs in two forms, with distinct morphologic features and different associated risks of neoplasia (Fig. 2 and 3). Many people in the general population are infected with HPV; the prevalence is as high as 10 to 15 percent in the younger, more sexually active population. In acute infections, a complete copy of the HPV viral DNA is present as an episome within the host cell, and the virus is capable of completing its life cycle, producing new, infectious viral particles. In the majority of cases, the infection is transient and results either in no demonstrable cytologic changes or in the cytologic changes associated with a productive viral infection. The presence of complete viral particles within the cell may cause a characteristic perinuclear clearing known as koilocytosis (Fig. 4A and 4B), which is the hallmark of the diagnosis of a low-grade squamous intraepi-
thelial lesion on Papanicolaou smears and corresponds to the histologic lesion on biopsy specimens classified as cervical intraepithelial neoplasia, grade 1. This lesion by itself is not associated with an increased risk of cervical neoplasia.

In a small minority of HPV-infected patients, the circular viral genome is integrated into the host-cell DNA, producing high-grade dysplasia, which can progress to invasive carcinoma (Fig. 3). During the integration process, the viral DNA is disrupted in the region of the E2 gene, which is responsible for controlling transcription of other viral genes. The oncopogenic genes E6 and E7, which encode proteins that bind to and inactivate the products of important host-cell tumor-suppressor genes, are preserved. Because of the loss of other viral genes during integration, the viral life cycle is not completed, and new virus particles are not produced. The cells of high-grade dysplasia (interpreted as high-grade squamous intraepithelial lesions on the Papanicolaou smear and typically reflected in biopsy findings of cervical intraepithelial neoplasia, grade 2 or 3, or invasive carcinoma) therefore do not show koilocytosis and are primitive and undifferentiated in appearance (Fig. 4C and 4D). It has been estimated that high-grade dysplasia may progress to invasive cancer in as many as 60 percent of cases if left untreated.

Given the central role of HPV in cervical carcinogenesis, testing for the presence of this virus can be an appropriate and cost-effective test to determine whether or not a patient with ASC-US, such as the patient in this case, may harbor high-grade dysplasia. There are several ways to test for the presence of HPV in clinical specimens. The most commonly...
are detected with the use of an enzyme-linked specimen, and the RNA–DNA complexes (hybrids) bind complementary DNA liberated from cells in a test. These probes have been reported; hence the theoretical sensitivity of less prevalent types are not included. Cross-reaction of some of the probes with low-risk viral types has been reported; hence the theoretical sensitivity and specificity are not 100 percent. The probes bind complementary DNA liberated from cells in a specimen, and the RNA–DNA complexes (hybrids) are detected with the use of an enzyme-linked chemiluminescent assay (hence the name “hybrid capture”).

The test can be performed on the residual cells present in a liquid-based cytologic specimen, in what is known as “reflex” testing. The test can also be performed with a specimen obtained with a cervical brush rinsed in transport medium specifically for the HPV test alone. The Hybrid Capture II HPV Test detects high-risk HPV in a highly sensitive manner, resulting in a very high negative predictive value for the detection of high-grade dysplasia. However, it is important to recognize that the large pool of patients who have transient viral infections without having high-grade dysplasia makes the specificity of the test low (i.e., makes the positive predictive value low).

Other tests available for the detection of high-risk HPV types are techniques based on the polymerase chain reaction and in situ hybridization. These tests theoretically have a very high sensitivity for viral detection and may also have the advantage of allowing specific viral typing. In situ hybridization combines the advantages of viral detection with simultaneous visualization of cellular morphologic features — a potential advantage for improving the specificity of the test for the detection of HPV-positive
cases of high-grade squamous intraepithelial lesions.

In addition to guiding management, HPV testing can also be useful in the laboratory for determining whether the designation of atypical squamous cells is being used properly. In addition to correlating such interpretations with the findings on follow-up biopsy specimens, laboratories can now determine the proportion of cases associated with high-risk HPV infection. It has been suggest-
ed that about 50 percent of patients whose smears are categorized as showing atypical squamous cells should be positive for HPV.

In the case under discussion, we tested a liquid-based cytologic specimen with the Hybrid Capture II HPV Test. The test was negative for the presence of high-risk HPV infections.

**DISCUSSION OF MANAGEMENT**

Dr. Goodman: A recent national study, sponsored by the National Cancer Institute, known as the ASCUS–LSIL Triage Study (where LSIL denotes low-grade squamous intraepithelial lesion), has provided information that is useful in determining the role of HPV testing in the care of patients with a Papanicolaou smear showing ASC-US. In this study, 3488 women with ASC-US on a single Papanicolaou smear were randomly assigned to one of three options: immediate colposcopy, HPV testing with the Hybrid Capture II HPV Test, or a repeated Papanicolaou test. Patients in whom HPV was detected by the Hybrid Capture II HPV Test and patients with a high-grade squamous intraepithelial lesion on the repeated Papanicolaou test were referred for colposcopy. All the patients underwent colposcopy at the end of the trial, with a repeated Papanicolaou test and biopsy of any suspicious areas.

HPV testing detected 92.4 percent of cases of cervical intraepithelial neoplasia, grade 3, whereas examination of the repeated Papanicolaou test, with high-grade squamous intraepithelial lesion used as the threshold for referral, detected only 54.6 percent. Fifty-six percent of the women who underwent HPV testing were referred for colposcopy, as compared with 12.3 percent of the women in the group assigned to a repeated Papanicolaou test. When the threshold for referral for colposcopy on the basis of a repeated Papanicolaou test was changed to any abnormal finding (ASC-US or higher, as is the current standard of practice), the sensitivity for the detection of cervical intraepithelial neoplasia, grade 3, increased to 95.4 percent but required two office visits and was associated with a referral rate for colposcopy of 67.1 percent. Thus, reflex HPV testing provides essentially equivalent sensitivity and slightly better specificity than a repeated Papanicolaou smear for detecting cervical intraepithelial neoplasia, grade 3, and eliminates the need for repeated Papanicolaou testing.

These data have led to the recommendation by the American Society for Colposcopy and Cervical Pathology that all women with Papanicolaou smears showing ASC-US should undergo HPV testing. Those whose tests are positive for high-risk HPV should then be referred for colposcopy. Those whose tests are negative should undergo repeated Papanicolaou testing in one year. Patients who have high-risk HPV and whose colposcopic evaluation shows no abnormalities should undergo repeated Papanicolaou testing at 6 and 12 months. Repeating the HPV testing after 12 months should also be considered for these patients, since persons with persistent HPV infections are at higher risk for the development of cervical neoplasia in the future than those with transient infections. This test will help determine the course of management more effectively than the Papanicolaou test alone and help reduce unnecessary testing in women whose HPV tests are negative.

At present, the only indication for HPV testing is a Papanicolaou smear showing ASC-US. However, the Food and Drug Administration recently approved HPV testing in conjunction with Papanicolaou testing for primary screening in women older than 30 years of age. Although infection with a high-risk type of HPV is a necessary condition for the development of lower genital tract cancer, it is clearly not sufficient, since more than 90 percent of HPV infections resolve completely within two years without the identification of clinically significant lesions. Thus, testing as part of primary screening in young patients may increase the frequency of detection of transient infections.

The patient under discussion had a negative test for HPV and is therefore at low risk for the development of cervical neoplasia. She was referred back to her primary care physician and will need no more than a routine yearly Papanicolaou test.

A Physician: The number of sexual partners that put a woman at risk for HPV-related disease has been variably defined as two to five or more. What do you consider to be an indicator of high risk?

Dr. Goodman: Exposure to multiple sexual partners is an indirect measure of risk, since the probability of contact with a high-risk HPV subtype increases with additional exposures. However, if the only sexual contact a woman has had is with a partner who harbors a high-risk HPV subtype, she is at risk. Once HPV testing becomes routine for the evaluation of Papanicolaou smears showing ASC-US, it will be clinically irrelevant to determine the number of sexual partners a woman has had.

A Physician: Will the HPV test be performed rou...
three doses of HPV-16 vaccine or a placebo. Years of age who were randomly assigned to receive a prospective trial involving 1533 women 16 to 23 years of age. Koutsky and colleagues reported the results of a randomized trial on the management of cervical cytology: the 1992 National Cancer Institute Workshop. JAMA 1994;271:1866-9.

A Physician: I have a patient in her early 40s whose Papanicolaou smear shows the over 5 years have shown ASC-US. She has had multiple colposcopic examinations followed by biopsies, which showed no abnormalities, and has had negative tests for high-risk HPV. At what point can we be certain that these Papanicolaou-smear findings do not represent high-grade dysplasia?

A Physician: I have an 80-year-old patient who has had two sexual partners in her lifetime and who has not been sexually active for 20 years. This year, her Papanicolaou smear showed a high-grade squamous intraepithelial lesion. The findings on a colposcopic examination and examination of a cervical-biopsy specimen were normal. Is there a role for HPV testing?

A Physician: This patient has a highly abnormal Papanicolaou smear with clear evidence of dysplasia. HPV testing in this setting is not indicated. She may have a lesion in her upper endocervix that is not apparent on colposcopy. A cervical cone biopsy would be an appropriate next step. It also will be important to evaluate her vagina and vulva carefully for other possible sources of the dysplastic cells.

**Pathological Diagnosis**

Atypical squamous cells of unknown significance (on the Papanicolaou smear), with a negative test for high-risk human papillomavirus.

The patient is currently not at increased risk for cervical cancer.

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