

**XIAN WEN JIN, MD, PhD**Department of General Internal Medicine,
The Cleveland Clinic**CRAIG NIELSEN, MD**Department of General Internal Medicine,
The Cleveland Clinic**JENNIFER BRAINARD, MD**Department of Anatomical Pathology,
The Cleveland Clinic**BELINDA YEN-LIEBERMAN, PhD**

Department of Clinical Pathology, The Cleveland Clinic

New advances transform the management of women with abnormal Pap tests

ABSTRACT

New advances in Papanicolaou test technology, human papillomavirus DNA testing, and revisions in the Bethesda terminology for cervical cytology have transformed the management of abnormal Pap tests. This approach has been validated by a recent randomized clinical trial, and in some instances can reduce the number of colposcopies by 50%.

KEY POINTS

The new liquid-collection, thin-layer system has improved the quality of Pap smears and the test's sensitivity for diagnosing precancerous cervical lesions.

The new Bethesda terminology has clarified the previous ambiguous reporting categories; the category of "atypical squamous cells of unknown significance" (ASCUS) has been divided into two: ASC-US and a higher-risk category called ASC-H.

Testing for the DNA of high-risk types of human papillomavirus (HPV) by the Hybrid Capture 2 assay provides a basis for deciding whether women with ASCUS should undergo colposcopy.

The ALTS trial clinically validated the value of HPV DNA testing in identifying women at risk for cervical cancer.

Women with ASC-US should be tested for HPV and undergo colposcopy if positive. Those with ASC-H should be referred for immediate colposcopy.

REMARKABLE ADVANCES in technology and in understanding of the development of cervical cancer have opened a new era in cervical cancer screening. In the past few years:

- The traditional technique of smearing cervical cells on a glass slide for the Papanicolaou (Pap) test has been largely replaced by a liquid-collection technique
- A major trial sponsored by the National Cancer Institute provided long-awaited data validating the utility of testing for human papillomavirus (HPV) in women with abnormal Pap smears
- An expert group of clinicians, pathologists, and researchers revised the Bethesda system terminology used for reporting results of cervical cytology screening
- New guidelines for managing women with abnormal Pap smears have been published that are based on the trial data and the new reporting system.

This article briefly summarizes the new liquid-collection system for thin-layer slide preparation, the new Hybrid Capture 2 HPV DNA test, the 2001 Bethesda reporting system, data from a key trial, and the new management guidelines.

SCOPE OF THE PROBLEM

Cervical lesions pose a major public health problem due to the enormous resources expended for preventing, diagnosing, and managing them.

Cervical cancer is the second leading cause of cancer deaths in women worldwide, with approximately 500,000 deaths per year.¹

In the United States, an estimated 13,000 women contract it each year and 4,100 die of it.^{2,3}

Since its introduction in the 1940s, screening with the Pap smear has reduced the mortality rate from cervical cancer by as much as 70%.^{4,5} However, many Pap smears are reported as “atypical squamous cells of unknown significance” (ASCUS), an ambiguous finding. Of the 50 million women undergoing Pap testing each year, about 3 million are diagnosed with ASCUS and require further evaluation.⁶

The cost of colposcopic evaluation of these lesions approaches \$6 billion annually—a substantial financial burden to the health care system. They also impose a heavy emotional burden on the women affected.⁷

Moreover, the Pap smear has a high false-negative rate, demonstrated in numerous studies to range from 10% to 50%.^{8–10}

■ NEW PAP TEST TECHNIQUE

Up to 90% of false-negative Pap smears are due to limitations of sampling or slide preparation. Accurate interpretation can be hindered by blood, mucus, inflammation, air-drying artifact, or areas of thick cellularity, all of which are common in conventional Pap smears.¹¹

To address these problems, a new slide preparation method—the liquid-collection ThinPrep system—was developed by the Cytoc Corporation (Boxborough, Mass) and approved by the US Food and Drug Administration in May 1996.¹²

How the ThinPrep system works

In the ThinPrep system, cells are placed immediately into a fixative (the PreservCyt liquid cytology medium) and transported to the laboratory, where thin-layer slides are prepared using the ThinPrep processor. The machine spins and filters the samples until sufficient cells have been obtained. The cells are then transferred from the filter to a glass slide for Pap staining.

The resulting slide contains approximately 50,000 cells, evenly distributed, representing 5% of the total number of cells in the collection in most cases. Sometimes the percentage is higher, eg, in postmenopausal women.

The new system is better

This new Pap test technique has resulted in a statistically significant increase in the cytologic diagnosis of cervical cancer precursors and in specimen adequacy.¹³ The clarity of the specimen increases the ease of screening by cytology technologists.

Most studies have found the liquid-based ThinPrep system to be more sensitive than conventional smears, while its specificity was comparable or a little less.

In addition, the captured and preserved cells can later be used for HPV DNA testing. This offers a cost-effective single-sample approach.

The ThinPrep technique has been adopted by most clinics in the United States. It has been the standard of care for Pap testing at The Cleveland Clinic since 1999. The cost is approximately \$80.

■ NEW HPV DNA TESTING BY HYBRID CAPTURE ASSAY

Cervical cancer is strongly associated with HPV infection. Between 93% and 100% of squamous cell carcinomas of the cervix contain DNA from high-risk types of HPV.

Testing for HPV DNA has greater sensitivity than cytology for detecting clinically relevant lesions, and it has been a useful adjunct test in cervical cancer screening since the mid-1990s.

In April 1999, the FDA approved a breakthrough technology, the Hybrid Capture 2 HPV DNA test (Digene Corporation; Gaithersburg, Md).

How the Hybrid Capture 2 test works

The new test is a sandwich capture molecular hybridization assay that utilizes chemiluminescence to detect one or more of the 13 cancer-associated (high-risk) HPV types, ie, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.¹⁴

Cervical specimens from a ThinPrep collection containing the target DNA hybridize in solution with a specific HPV RNA probe cocktail. The resulting RNA-DNA hybrids are captured onto the surface of a microplate well that is coated with an anti-RNA-DNA hybrid antibody.

The false-negative rate of traditional Pap tests is as high as 50%



TABLE 1

The 2001 Bethesda system for reporting Pap smear results (abridged)

SPECIMEN ADEQUACY

- Satisfactory for evaluation (note presence or absence of endocervical/transformation zone component)
- Unsatisfactory for evaluation (specify reason)
 - Specimen rejected or not processed (specify reason)
 - Specimen processed and examined but unsatisfactory for evaluation of epithelial abnormality (specify reason)

GENERAL CATEGORIZATION (optional)

- Negative for intraepithelial lesion or malignancy
- Epithelial cell abnormality
- Other

INTERPRETATION/RESULT

Negative for intraepithelial lesion or malignancy

- Organisms
 - Trichomonas vaginalis*
 - Fungal organisms morphologically consistent with *Candida* species
 - Shift in flora suggestive of bacterial vaginosis
 - Bacteria morphologically consistent with *Actinomyces* species
 - Cellular changes consistent with herpes simplex virus
- Other nonneoplastic findings (optional to report; list not comprehensive)
 - Reactive cellular changes associated with:
 - Inflammation (includes typical repair)
 - Radiation
 - Intrauterine contraceptive device
 - Glandular cells status post hysterectomy
 - Atrophy

Epithelial cell abnormalities

- Squamous cell
 - Atypical squamous cells (ASC)
 - Of undetermined significance (ASC-US)
 - Cannot exclude HSIL (ASC-H)
 - Low-grade squamous intraepithelial lesion (LSIL)
 - Encompassing: human papillomavirus, mild dysplasia, cervical intraepithelial neoplasia (CIN 1)
 - High-grade squamous intraepithelial lesion (HSIL)
 - Encompassing: moderate and severe dysplasia, carcinoma in situ, CIN 2, CIN 3
 - Squamous cell carcinoma
- Glandular cell
 - Atypical glandular cells (AGC) (specify endocervical, endometrial, or not otherwise specified)
 - Atypical glandular cells, favor neoplastic (specify endocervical or not otherwise specified)
 - Endocervical adenocarcinoma in situ (AIS)
 - Adenocarcinoma
- Other (list not comprehensive)
 - Endometrial cells in a woman ≥ 40 years of age

AUTOMATED REVIEW AND ANCILLARY TESTING (include as appropriate)

EDUCATIONAL NOTES AND SUGGESTIONS (optional)

FROM SOLOMON D, DAVEY D, KURMAN R, ET AL. THE 2001 BETHESDA SYSTEM: TERMINOLOGY FOR REPORTING RESULTS OF CERVICAL CYTOLOGY. JAMA 2002; 287:2114–2119.

Captured hybrid is then reacted with an antihybrid antibody conjugated to alkaline phosphatase and detected with a chemiluminescent substrate.

When the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured in relative light units on a luminometer. The intensity of the light emitted is proportional to the amount of HPV DNA in the Pap specimen.

Highly sensitive

The new Hybrid Capture 2 test has a high diagnostic sensitivity (85% to 100%) and negative predictive value (99% to 100%).^{14,15} The specificity is about 61%. Older tests were less sensitive and specific.

■ NEW PAP TEST TERMINOLOGY

Before 2001, more than 90% of US laboratories used some form of the 1991 Bethesda system in reporting cervical cytology.¹⁶ This provided a standardized framework for reporting a descriptive diagnosis that clinicians could use to make treatment decisions. With the advances in new technologies, recent findings from research studies, and the latest information from clinical trials, the Bethesda system was revised in 2001.

Eight months before the Bethesda 2001 workshop, nine forum groups were established to draft recommendations for modifying the Bethesda system. A Web site with an electronic bulletin board was set up to seek input, and more than 1,000 comments were collected. More than 400 experts, including pathologists, cytotechnologists, clinicians, and patient advocates participated in the workshop.

The 2001 Bethesda system was published in the April 24, 2002, issue of JAMA, the Journal of the American Medical Association (TABLE 1),¹⁷ and more than 20 national and international societies have endorsed it.

Changes in the 2001 Bethesda system

The 2001 Bethesda System contains several important changes.

- It eliminates the category “benign cellular changes,” which had generated confusion about whether women in this category were at higher risk of cervical cancer. In the 2001

updated version, benign changes are more clearly identified as “negative for intraepithelial lesion or malignancy.”

- It also removes the phrases “favor reactive processes” and “favor benign processes” in ambiguous findings.
- Probably the most important change: the previous equivocal category of ASCUS has been subdivided into two groups:

ASC-US: atypical squamous cells of undetermined significance

ASC-H: atypical squamous cells, cannot exclude a high-grade lesion.

Women with the ASC-H Pap diagnosis are at higher risk of ultimately developing a high-grade lesion and should be referred for colposcopy.

Women with ASC-US are at a lower risk of developing a high-grade lesion than those with ASC-H, but they are at a higher risk than those diagnosed “negative for intraepithelial lesion.”

This distinction provides further risk stratification in this subset of patients and the foundation for clinical decision-making.¹⁸

■ NEW EVIDENCE FROM A CLINICAL TRIAL

A major issue in cervical cancer screening was how to manage the 3 million women diagnosed with ASCUS each year. Most of these mild cervical abnormalities regress spontaneously without treatment, but physicians had no way to identify clinically significant lesions that represent precancer or cancer and that need treatment.

To clarify the management of mildly abnormal cervical cytopathology, the National Cancer Institute in 1996 launched a major randomized, multicenter clinical trial known as the Atypical Squamous Cells of Undetermined Significance/Low-grade Squamous Intraepithelial Lesions (ASCUS/SIL) Triage Study (ALTS).^{19–21}

From 1996 to 1998, a total of 5,060 women with abnormal cervical cytologic findings (ASCUS or LSIL) enrolled at four sites. All underwent HPV testing by Hybrid Capture 2 assay.

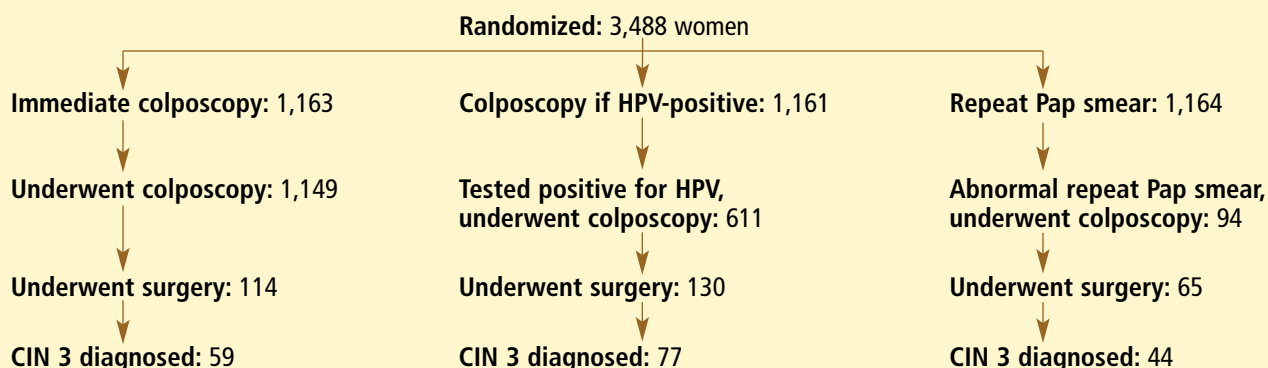
Results in women with LSIL

Among 642 women with low-grade squamous

HPV tests can be done from the same ThinPrep sample used for cytology



HPV testing in women with ASCUS: Results from a trial



HPV testing = testing for DNA from high-risk strains of human papillomavirus by Hybrid Capture 2 assay; ASCUS = atypical squamous cells of undetermined significance; Pap = Papanicolaou; CIN 3 = cervical intraepithelial neoplasia grade 3

FROM SOLOMON D, SCHIFFMAN M, TARONE R. COMPARISON OF THREE MANAGEMENT STRATEGIES FOR PATIENTS WITH ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE: BASELINE RESULTS FROM A RANDOMIZED TRIAL. J NATL CANCER INST 2001; 93:293-299.

FIGURE 1

intraepithelial lesions, high-risk HPV DNA was detected in 532 (82.9%).

The high percentage of HPV DNA positivity in the LSIL population limits the usefulness of HPV testing in clinical decision-making. It was estimated that the cost of HPV testing of all women with a cytologic diagnosis of LSIL would outweigh savings gained from avoiding colposcopy for only 20% to 27% of women.

Therefore, in October 1997, the ALTS steering committee decided that women with LSIL would no longer be randomly assigned to a follow-up protocol that used HPV DNA results for triage.¹⁹

Results in women with ASCUS

A total of 3,488 women with ASCUS were randomly assigned to one of three management strategies (FIGURE 1):

- Immediate colposcopy
- Colposcopy if HPV-positive (HPV test triage).
- Repeat Pap smear

The primary end point of the study was histologically confirmed cervical intraepithelial neoplasia grade 3 (CIN 3).

High-risk HPV DNA was detected in 1,766 (50.6%) of the 3,488 participants. Overall, 5.1% of women with ASCUS in the

trial had histologically confirmed CIN 3. The sensitivity of the HPV DNA test for predicting CIN 3 or cancer was 96.3%, with a negative predictive value of 99.5%. In contrast, the sensitivity of a single repeat Pap test was only 44.1%. About 55% of women with ASCUS would have been referred for colposcopy if the HPV test had been used for triage in all cases.

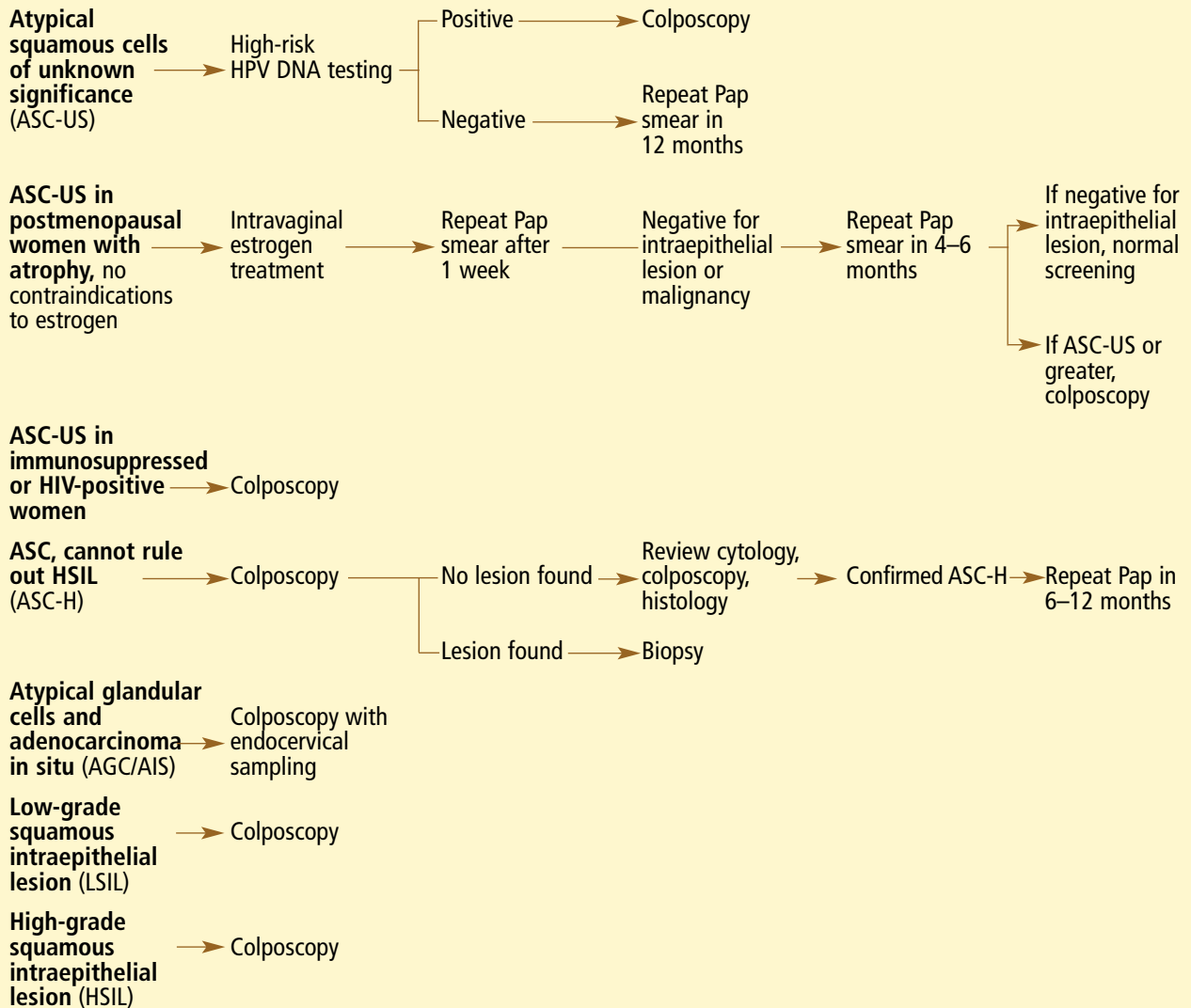
This trial shows that the Hybrid Capture 2 assay has excellent sensitivity for detecting precancerous cervical lesions. HPV testing can help in deciding how to manage women with ASCUS. A positive test suggests that precancer or (rarely) cancer may be present, while a negative test may assure women of the benign nature of their ASCUS. Precancerous lesions were found in 13% of ASCUS cases in which the HPV test was positive. HPV testing reduced referrals to colposcopy by about 50% compared with immediate colposcopy. Thus, HPV testing is a viable option in triaging women with ASCUS.²⁰

NEW GUIDELINES FOR ABNORMAL PAP SMEARS

Pathologists and clinicians have long struggled to deal with the diagnosis of ASCUS, since

**Only about 5%
of cases of
ASCUS are CIN 3**

An algorithm for managing abnormal Pap smear results



BASED ON WRIGHT TC JR, COX JT, MASSAD LS, TWIGGS LB, WILKINSON EJ. 2001 CONSENSUS GUIDELINES FOR THE MANAGEMENT OF WOMEN WITH CERVICAL CYTOLOGICAL ABNORMALITIES. JAMA 2002; 287:2120-2129.

FIGURE 2

the significance to the patient was uncertain. Ignoring ASCUS is clearly dangerous, yet referring all women with ASCUS for immediate colposcopy is costly and unnecessary.

The convergence of remarkable events in 2001 provided pivotal information needed for a comprehensive evidence-based management guideline for women with ASCUS or LSIL. The ALTS investigators published their baseline results in March 2000.¹⁹ The long-awaited data validating the utility of HPV testing in triaging women with ASCUS was published

in January 2001.²⁰

Both the ALTS trial data and the new Bethesda system terminology provided the framework for a comprehensive, evidence-based guideline for the management of women with abnormal Pap smears.

From September 6th through September 8th, 2001, the American Society for Colposcopy and Cervical Pathology (ASCCP) hosted a consensus conference in Bethesda, Md, to develop the guidelines. Representatives from 29 professional health organizations and



federal agencies participated in the conference. After comprehensive discussion, revision, and voting, an evidence-based consensus guideline was developed.²² The recommendations for managing women with abnormal cervical cytology are summarized in **FIGURE 2**.

Women with ASC-US should be tested for high-risk HPV DNA. Those testing positive are referred for colposcopy. Women with ASC-US who test negative for high-risk HPV should repeat a Pap test at 1 year.

Women with ASC-H, LSIL, HSIL, (high-grade squamous intraepithelial lesion), or **atypical glandular cells** should be referred for immediate colposcopic evaluation.

HPV testing in women with ASCUS should reduce the colposcopy referral rate by 50%. However, only about 5% of cases of ASCUS reflect an underlying CIN 3, an immediate cancer precursor. The less-than-ideal specificity of the high-risk HPV DNA test would prevent it from replacing the Pap test in primary screening. Therefore, many women with ASCUS will still undergo unneeded colposcopy.

A recent study indicated that the prevalence of high-risk HPV infection declines with age: only 31.2% among women with ASCUS who were 29 years or older, compared with 65% in those age 28 and younger.²³ Thus, the reduced referrals with HPV DNA testing in older women may be promising in evaluating the cost-effectiveness of HPV triage and further improving strategies for ASCUS management.

More recently, the American Cancer Society²⁴ recommended considering that cervical cancer screening be performed every 3 years using conventional or liquid-based cytology combined with a test for high-risk HPV DNA in women age 30 and over.²⁴ However, the FDA has not yet approved the use of HPV DNA testing with cytology for primary cervical cancer screening.

The clinical value of HPV testing for deciding who should undergo colposcopy depends on several factors: the sensitivity and specificity of the test, the prevalence of disease in different age groups, and the cost-effectiveness of the procedure. The positive predictive value of HPV testing may be further influenced by infections with different HPV types (high-risk types vs intermediate-risk types), the number of atypical cells, and other factors. Further studies will help to provide insights into how to improve predictive power, improve outcomes, and reduce costs.

■ A CERVICAL CANCER VACCINE

Recently, Koutsky et al²⁵ concluded a clinical trial of a vaccine against HPV-16. Vaccinated women were protected not only from preinvasive disease associated with HPV-16 but also from persistent and transient HPV-16 infection, with efficacy rates of 100%, 100%, and 91%, respectively.

A successful vaccine against all high-risk HPV strains will mark the beginning of the eradication of cervical cancer. 

HPV testing could reduce referrals for colposcopy by 50%

■ REFERENCES

1. Wright TC, Ferenczy A, Kuman RJ. Carcinoma and other tumors of the cervix. In: Kurman RJ, ed. *Pathology of the Female Genital Tract*. 4th ed. New York: Springer Verlag; 1994:279–326.
2. Center for Disease Control and Prevention. www.cdc.gov/nccdphp/brf-ss/index.htm.
3. National Cancer Institute. Cervical Cancer Screening—Health Professionals. www.cancer.gov. May 2002.
4. Devesa SS, Silverman DT, Young JL, et al. Cancer incidence and mortality trends among whites in the United States, 1947–84. *J Natl Cancer Inst* 1987; 79:701–770.
5. National Cancer Institute. Cancer Statistics Review 1973–1987. 1990 Bethesda, MD. Report No.: NCI Pub. No. (NIH) 90-2789.
6. Jones BA, Davey DD. Quality management in gynecologic cytology using interlaboratory comparison. *Arch Pathol Lab Med* 2000; 124:672–681.
7. Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman MH. Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. *JAMA* 1994; 271:1866–1869.
8. Dehner LP. Cervicovaginal cytology, false-negative results, and standards of practice. *Am J Clin Pathol* 1993; 99:45–47.
9. Gay JD, Donaldson LD, Goellner JR. False-negative results in cervical cytologic studies. *Acta Cytol* 1985; 29:1043–1046.
10. Dodd LG, Sneige N, Villarreal Y, et al. Quality-assurance study of simultaneously sampled, non-correlating cervical cytology and biopsies. *Diagn Cytopathol* 1993; 9:138–144.
11. McGoogan E, Reith A. Would monolayers provide more representative samples and improved preparations for cervical screening? Overview and evaluation of systems available. *Acta Cytol* 1996; 40:107–119.
12. Corkill M, Knapp D, Hutchinson ML. Improved accuracy for cervical cytology with the thin prep method and the endocervical brush-spatula collection procedure. *Journal of Lower Genital Tract Disease* 1998;2:12–16.
13. Lee KR, Ashfaq R, Birdsong GG, Corkill ME, McIntosh KM, Inhorn SL. Comparison of conventional Papanicolaou



- smears and a fluid-based, thin-layer system for cervical cancer screening. *Obstet Gynecol* 1997; 90:278–284.
14. **Manos MM, Kinney WK, Hurley LB, et al.** Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results. *JAMA* 1999; 281:1605–1610.
 15. **Nindl I, Lorincz A, Mielzynska I, et al.** Human papillomavirus detection in cervical intraepithelial neoplasia by the second-generation hybrid capture microplate test, comparing two different cervical specimen collection methods. *Clin Diagn Virol* 1998; 10:49–56.
 16. **Davey DD, Woodhouse S, Styer P, Stastny J, Mody D.** Atypical epithelial cells and specimen adequacy: current laboratory practices of participants in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med* 2000; 124:203–211.
 17. **Solomon D, Davey D, Kurman R, et al.** The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 2002; 287:2114–2119.
 18. **Stoler MH.** New Bethesda terminology and evidence-based management guidelines for cervical cytology findings. *JAMA* 2002; 287:2140–2141.
 19. **The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group.** Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. *J Natl Cancer Inst* 2000; 92:397–402.
 20. **Solomon D, Schiffman M, Tarone R.** Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst* 2001; 93:293–299.
 21. **Stoler MH, Schiffman M.** Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA* 2001; 285:1500–1505.
 22. **Wright TC Jr, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ.** 2001 Consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002; 287:2120–2129.
 23. **Sherman ME, Schiffman M, Cox JT.** Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS). *J Natl Cancer Inst* 2002; 94:102–107.
 24. **Saslow D, Runowicz CD, Solomon D, et al.** American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin* 2002; 52:342–362.
 25. **Koutsky LA, Ault KA, Wheeler CM, et al.** A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002; 347:1645–1651.

ADDRESS: Xian Wen Jin, MD, PhD, Department of General Internal Medicine, S70, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195; e-mail jinx@ccf.org.

Visit our web site at
<http://www.ccjm.org>

Contact us by e-mail at
ccjm@ccf.org