ProEx C Immunocytochemistry and High-Risk Human Papillomavirus DNA Testing in Papanicolaou Tests With Atypical Squamous Cell (ASC-US) Cytology

Correlation Study With Histologic Biopsy

Momin T. Siddiqui, MD, FIAC; Kelly Hornaman, MD; Cynthia Cohen, MD; Aziza Nassar, MD, FIAC

● ***Context.*—Papanicolaou tests with atypical squamous cells of undetermined significance (ASC-US) cytology and adjunct testing for high-risk human papillomaviruses (hr-HPV) are helpful in detecting high-grade disease. Detection of disease may be further improved with molecular markers known to be overexpressed in cervical carcinoma. ProEx C detects 2 such molecular markers, minichromosome maintenance protein 2 and topoisomerase II, which are associated with abnormal cell cycle regulation.**

***Objective.*—To determine the utility of ProEx C as a marker for high-grade cervical intraepithelial neoplasia 2+ disease when compared with hr-HPV status in Papanicolaou tests with ASC-US cytology.**

***Design.*—A SurePath slide was prepared on all ASC-US cases from the residual SurePath vial pellet and stained using the ProEx C reagent prediluted with water-bath antigen retrieval, using a Dako autostainer. Nuclear staining of cytologically atypical squamous cells was considered a positive result. Adjunct testing for hr-HPV used Digene Hybrid Capture 2. Follow-up biopsy results were available for review following the Papanicolaou test.**

***Results.*—Two hundred patients with ASC-US diagnoses were part of this study. The sensitivities of ProEx C and hr-HPV testing in detecting high-grade cervical intraepithelial neoplasia 2+ disease were 98.04% and 82.35%, respectively, whereas the specificity for detecting high-grade disease was 74.50% and 73.15%, respectively.**

***Conclusions.*—ProEx C staining is a more sensitive and specific biomarker for detecting cervical disease than adjunct testing for hr-HPV status in Papanicolaou tests with ASC-US.**

(*Arch Pathol Lab Med.* 2008;132:1648–1652)

The advent of the Papanicolaou (Pap) test has resulted in the increased detection of cervical carcinoma and has resulted in decreased morbidity and mortality from these malignancies in female patients.[1] However, cervical carcinoma is still the second most common malignancy in women worldwide and is the most common female malignancy in developing countries.[2] In the year 2000, approximately 450 000 women worldwide developed cervical carcinoma, and more than 200 000 died from the disease.[2] More than 50% of these cervical carcinoma patients were never screened, and of these unscreened patients, 10% to 20% were not screened in the previous 5 years.[3–5] Approximately 30% of cancer patients had at least 1 false-negative Pap test because of errors in either sampling or cytologic interpretation before the development of invasive cervical carcinoma.[6] Thus, any single Pap test event is only 50% sensitive for detecting high-grade squamous intraepithelial lesion (HSIL) or invasive carcinoma.[7] Although independent, recurring Pap tests contribute to a successful screening program, nevertheless, even with perfect compliance, the system has its limitations because of less-than-ideal sensitivity, interpretive variance, and morphologic subjectivity.

The Atypical Squamous Cells of Undetermined Significance (ASC-US)–Low Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS) found that 12% of women with positive ASC-US cytologic results had an underlying high-grade squamous intraepithelial lesion (HSIL).[8,9] This finding would result in an estimated 3 million American women receiving abnormal Pap test results that would require colposcopy to exclude HSIL.[10]

The limitations encountered with the Pap test are now beginning to be addressed by ancillary molecular testing of cervical/vaginal samples. High-risk human papillomavirus (hr-HPV) DNA testing can separate women with an ASC-US diagnosis into 2 groups, with each group having a different risk for HSIL. One group has negative results for hr-HPV DNA and carries a very low risk of HSIL, similar to the risk for a woman with negative results for intraepithelial lesion.[11] Hence, a woman with a negative finding for intraepithelial lesion on the Pap test and a negative result from an HPV DNA test is at very low risk of HSIL and can have her screening interval increased.[12–14] Women in the other group, with positive results from an

Accepted for publication March 12, 2008.

From the Department of Pathology and Laboratory Medicine, Emory University Hospital, Atlanta, Ga (Drs Siddiqui, Hornaman, and Cohen); and the Department of Pathology, Mayo Clinic, Rochester, Minn (Dr Nassar).

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Momin T. Siddiqui, MD, FIAC, Department of Pathology and Laboratory Medicine, Emory University Hospital, 1364 Clifton Rd NE, Atlanta, GA 30322 (e-mail: Momin.siddiqui@emoryhealthcare.org).

HPV DNA test, however, have an increased likelihood of HSIL.[15]

The most helpful molecular marker for HSIL should have the negative predictive value of a negative HPV DNA test and a higher positive predictive value. One such biomarker being studied is ProEx C (Tripath Imaging, Burlington, NC), which is an immunocytochemical assay that targets the expression of topoisomerase II-α (TOP2A) and minichromosome maintenance protein 2 (MCM2).[16,17] Both *TOP2A* and *MCM2* have been identified by transcriptional profiling as genes that are overexpressed in cervical carcinoma.[18–20] Overexpression of *TOP2A* and *MCM2* is a result of aberrant S-phase induction. The HPV oncoproteins are directly involved in the development of cervical dysplasia and carcinoma, E6 through its interaction with tumor suppressor protein p53, and E7 through interaction with hypophosphorylated retinoblastoma proteins pRb, p107, and p130.[21] This induces the aberrant transcription of S-phase proteins, such as TOP2A and MCM2.[21] In DNA synthesis and proliferation, TOP2A causes enzymatic unlinking of DNA strands during replication, and MCM2 functions during DNA replication by loading the prereplication complex into DNA and unwinding DNA through helicase activity to permit DNA synthesis.[22]

The current study was undertaken to compare ProEx C immunocytochemical staining and the Hybrid Capture 2 hr-HPV DNA test (Digene Corporation, Gaithersburg, Md) in detecting high-grade cervical disease using liquid-based SurePath (Becton, Dickinson and Company, Franklin Lakes, NJ) cytologic specimens positive for ASC-US. Histologic biopsy follow-up was also available for review.

MATERIALS AND METHODS

Specimen Criteria and Collection

The institutional review board of the Emory University, Atlanta, Ga, approved this study protocol. Two hundred residual cervical/vaginal cytology specimens were collected in SurePath preservative for this study. Specimens were obtained from the Emory Cytopathology Laboratory in 2006 and 2007, after clinical evaluation was completed, and a diagnosis of ASC-US was rendered. The original clinical diagnosis was rendered by 4 board-certified cytopathologists on staff at Emory University Hospital. Two hundred consecutive diagnoses of ASC-US were tracked, and the SurePath preservative-vial residual pellet was retrieved within 48 hours for processing an additional unstained slide for ProEx C immunocytochemistry. Cervical/vaginal biopsy data on all 200 cases were available within 1 month for review because all ASC-US cases were being biopsied with patient consent in a separate clinical protocol approved by the institutional review board. A random biopsy of normal-appearing cervix or vagina was performed if no lesion was identified at colposcopy.

Hybrid Capture HPV DNA Test

The Hybrid Capture 2 hr-HPV DNA test (Digene) was performed on all 200 liquid-based SurePath cytologic specimens. The samples were received within 1 day of collection and stored at room temperature until testing was performed (within 2 weeks of specimen collection) according to the manufacturer's recommendations, using the high-risk probes with the positive and negative control samples run in triplicate and result validation using Hybrid Capture 2 software, version 2.0. The hr-HPV panel consisted of 13 types of HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Test results with relative light units/cutoff values of less than 1.0 were considered negative. A ratio between 1.0 and 2.5 was considered an equivocal result. A ratio greater than 2.5 was considered a positive result.

Table 1. The Follow-up Biopsy, ProEx C, and High-Risk Human Papillomavirus (hr-HPV) Test Results

Diagnostic Categories*	No. of Patients		
	Cervical Biopsy	ProEx C Positive	hr-HPV Positive
Negative for dysplasia	90	7	13
CIN 1	59	31	27
CIN 2	38	37	29
CIN 3	13	13	13

* CIN indicates cervical intraepithelial neoplasia; CIN 1, low-grade dysplasia; CIN 2, moderate dysplasia; and CIN 3, severe dysplasia and carcinoma in situ.

ProEx C Immunocytochemical Staining

SurePath-processed, unstained slides were stained with ProEx C, which is a class I, in vitro, immunocytochemical stain that is used with standard immunocytochemistry techniques to detect aberrant S-phase induction and which contains antibodies to TOP2A and MCM2 proteins. The SurePath-processed slides were prepared using the PrepStain (Becton, Dickinson) processor and were treated with a pretreatment buffer for target retrieval, using SureDetect (Becton, Dickinson) slide-preparation buffer.

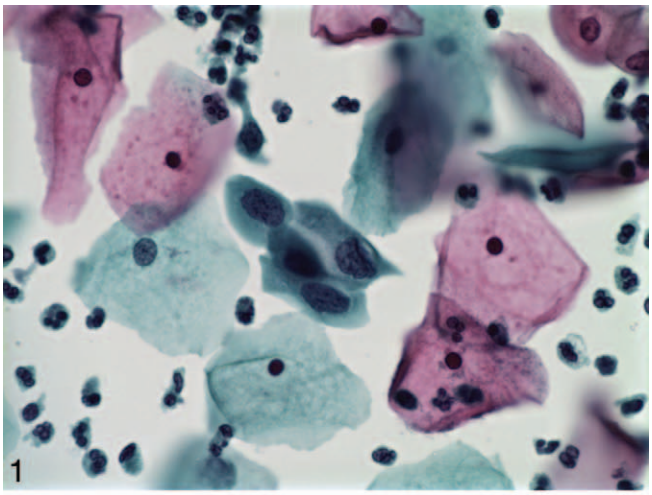
Immunocytochemical staining was performed with ProEx C, which is a detection reagent that includes a 3,3′-diaminobenzidine tetrahydrochloride–based chromogen and hematoxylin-based counterstain (SureDetect detection reagent and SureDetect counterstain), using an automated staining platform (Dako Autostainer, DakoCytomation, Glostrup, Denmark). The entire surface area of each specimen was evaluated to confirm the absence of nonspecific staining, to verify the nuclear staining of the ProEx C stain, and to review the positive control slides with SiHa cells. The slides were than scored by conventional microscopy.

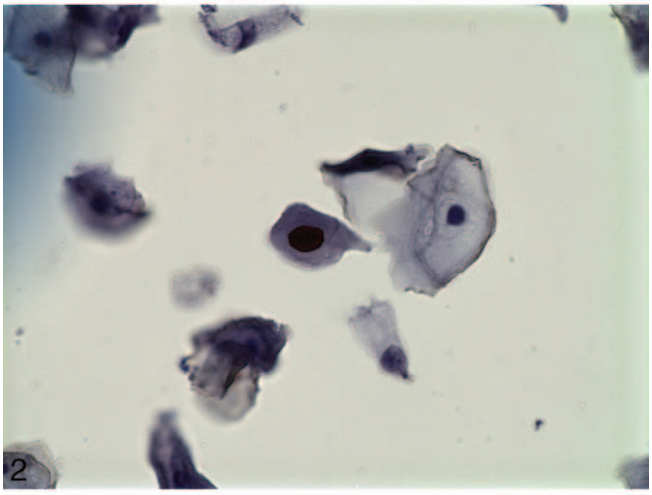
Slide-Scoring Algorithm

The slides were scored using a 3-step algorithm. The first step was to determine the adequacy of the specimen according to the 2001 Bethesda System for reporting cervical cytology criteria.[23] The second step was to determine whether there was brown nuclear staining in the squamous cells. The third and final step was to determine whether the stained cells showed abnormalities that conformed to the diagnostic criteria of ASC-US. If all 3 steps were affirmed, the slide was interpreted as positive, and likewise, if the 3 steps were not applicable, the slide was interpreted as negative.

RESULTS

Two hundred women (aged 17–57 years) with a diagnosis of ASC-US were part of the study. Results from the tests and from the follow-up cervical biopsies are summarized in Table 1. ProEx C staining was detected in 88 of the 200 ASC-US cases (Figures 1 through 3). Sporadic staining of benign-appearing endocervical cells was also noted in 92 of the cases reviewed; however, morphologically, these cells were not atypical and, hence, were not counted as positive test results (Figures 4 and 5). The cervical biopsy was used as a gold standard and based on it, the data for detection of high-grade disease were tabulated (Tables 1 and 2). The sensitivity for detecting high-grade (cervical intraepithelial neoplasia [CIN] 2+) disease by ProEx C and adjunct hr-HPV testing was 98.04% and 82.35%, respectively, whereas the specificity for detecting high-grade disease was 74.50% and 73.15%, respectively (Table 2). Both methods identified all cases of CIN 3 (Table 1). In addition, data for detection of overall (CIN 1+) disease were also tabulated (Table 3).





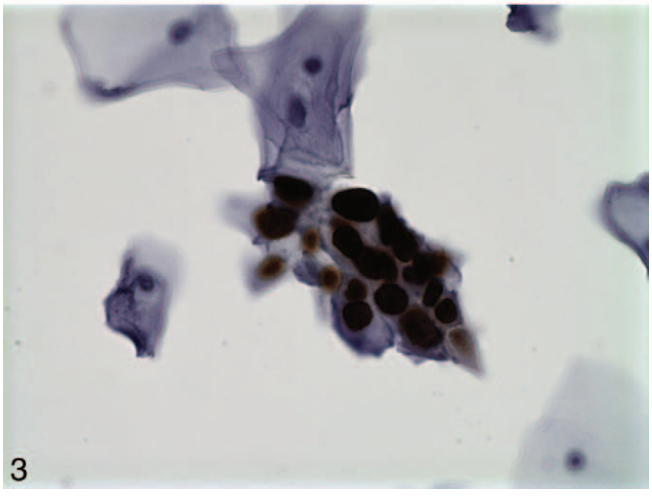
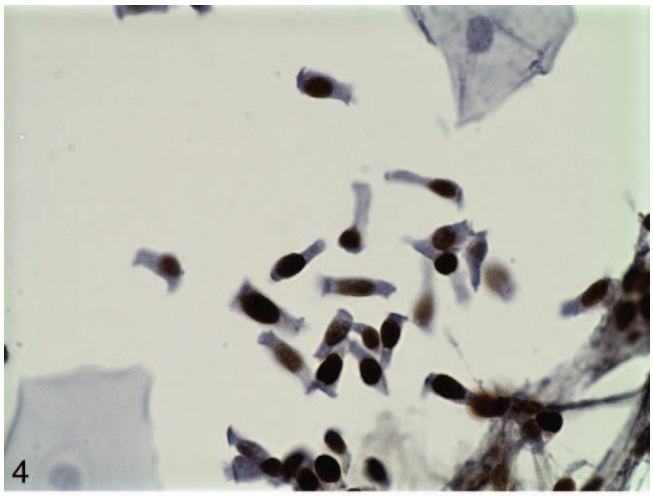


Figure 1. *Papanicolaou-stained atypical squamous cells of undetermined significance with background acute inflammation (original magnification ×600).*

Figure 2. *A single, atypical cell with morphologic features consistent with atypical squamous cells of undetermined significance showing dark nuclear staining with ProEx C (original magnification ×600).*

Figure 3. *A group of atypical squamous cells of undetermined significance showing positive nuclear staining with ProEx C (original magnification ×600).*



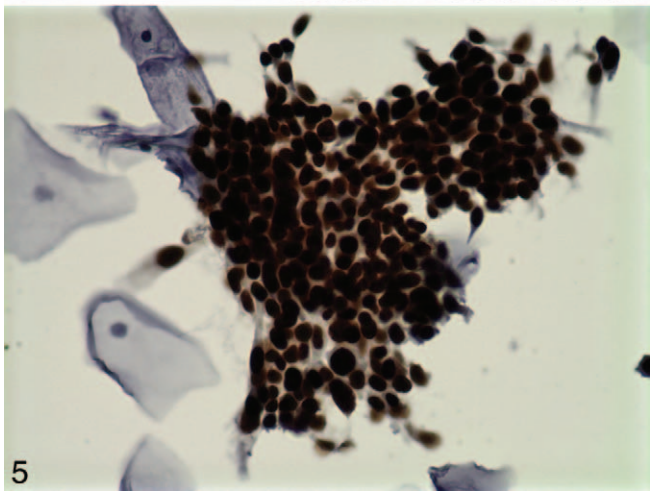


Figure 4. *Scattered, single, benign endocervical cells showing nuclear staining with ProEx C (original magnification ×600).*

Figure 5. *A group of reactive endocervical cells with ProEx C staining (original magnification ×600).*

COMMENT

In the United States, despite a highly successful Pap test screening program, an estimated 11 070 women will be diagnosed with cervical carcinoma in 2008.[24] In addition, more than 3 million women each year will receive an equivocal Pap test result.[25] This, depending on the clinical practice, may require HPV testing, colposcopy, or a cervical biopsy to exclude the presence of high-grade dysplasia or squamous cell carcinoma. In view of this background, the current study was conducted in patients with an ASC-US diagnosis and follow-up histologic biopsy to compare ProEx C and hr-HPV testing.

Human papillomavirus infection is an established etiology for development of squamous cell carcinoma.[26] The Digene Hybrid Capture 2 HPV DNA test is a molecular technique introduced for HPV detection in patients with ASC-US and LSIL diagnoses to identify patients with hr-HPV who are, therefore, more likely to progress to HSIL or squamous cell carcinoma.[27] This test, however, has limitations. A recent meta-analysis reported that the sensitivity and specificity for HPV DNA testing using the Hybrid Capture 2 HPV DNA test to detect high-grade cervical disease were 94.8% and 67.3%, respectively.[28] Another

Table 2. Comparison of Calculated Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Detection of High-Grade Cervical Intraepithelial Neoplasia (2+, Moderate Dysplasia) Disease With ProEx C and High-Risk Human Papillomavirus (hr-HPV) Testing

Evaluated Tests	Sensitivity, % (Range)	Specificity, % (Range)	PPV, % (Range)	NPV, % (Range)
ProEx C	98.04 (89.7–99.65)	74.50 (66.94–80.82)	56.82 (45.84–67.20)	99.11 (94.40–99.95)
hr-HPV	82.35 (69.75–90.43)	73.15 (65.52–79.62)	51.22 (40.01–62.32)	92.37 (85.62–96.23)

Table 3. Comparison of Calculated Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Detection of Cervical Intraepithelial Neoplasia (1+; Low-Grade Dysplasia) Disease With ProEx C and High-Risk Human Papillomavirus (hr-HPV) Testing

Evaluated Tests	Sensitivity, % (Range)	Specificity, % (Range)	PPV, % (Range)	NPV, % (Range)
ProEx C	73.64 (64.71–80.97)	92.22 (84.81–96.18)	92.05 (83.77–96.47)	74.11 (64.82–81.71)
hr-HPV	62.73 (53.41–71.19)	85.56 (76.84–91.36)	84.15 (74.05–90.96)	65.25 (55.87–73.63)

study reported a false-positive rate of 6.2% for HPV DNA testing when used in cervical cancer screening.[29] Additionally, the false-negative rate for the Hybrid Capture 2 HPV DNA test ranges from 3.7% to 18.2%.[28–30] In view of this data, additional molecular markers need to be studied to explore their utility in detecting high-grade cervical disease.

ProEx C is a new immunocytochemical marker that can offer an alternative or adjunct test in an equivocal Pap test result and can help detect high-grade disease.[17] In a recent study,[16] this marker was successfully validated in a retrospective analysis to determine its positive predictive value (PPV) in biopsies of proven cases of HSIL. In that study cohort, the PPV for ProEx C was 44.6% in patients with an ASC-US diagnosis, and 100% of the HSIL samples, 25% of LSIL samples, and 30% of samples from women that were concordant on LSIL cytology and CIN 1 biopsy were reported as positive. Therefore, in the absence of a biopsy as proof of high-grade disease, positive ProEx C results in patients with LSIL indicate MCM2 and TOP2A overexpression in LSIL cells, and a closer follow-up may be warranted in such patients because of increased risk for developing a high-grade lesion in the future.

Shroyer et al[31] have also reviewed the utility of ProEx C in cervical cytology in a recent 2-pronged study with an initial component that included validation of ProEx C in normal versus SiHa-spiked cells and HSIL test cases. SiHa cells are a control cell line derived from a patient with American Joint Committee on Cancer grade 2 squamous cell carcinoma of the cervix and is reported to contain 1 to 2 copies of the integrated HPV-16 genome per cell. ProEx C demonstrated 100% sensitivity and specificity for the classification of slides that showed positive or negative results for SiHa or HSIL cells. The second component of that study[31] involved evaluating 40 clinical samples, which included 10 cases each with a negative diagnosis for intraepithelial lesion, ASC-US, LSIL, and HSIL. ProEx C staining was uniformly absent in all cases with a diagnosis of intraepithelial lesion and uniformly positive in all cases with a diagnosis of HSIL. ASC-US and LSIL cases showed ProEx C positivity in 20% and 50% of the cases, respectively. However, the results from the populations with ASC-US and LSIL lacked biopsy correlation.

Our study included 200 patients with a diagnosis of ASC-US, and had subsequent ProEx C and hr-HPV testing and follow-up biopsy. Of the 200 patients, 51 (25.5%) had high-grade (CIN 2+) disease. The cervical biopsy was used as a gold standard, and based on it, the sensitivities for ProEx C and hr-HPV were 98.04% and 82.35%, respectively, whereas the specificity was 74.50% and 73.15%, respectively, for the detection of high-grade (CIN 2+) disease. An additional 59 (29.5%) of the 200 patients had low-grade dysplasia (CIN 1). The overall detection rate for cervical disease (CIN 1+) showed a sensitivity of 73.64% and 62.73% for ProEx C and hr-HPV, respectively, whereas the specificity was 92.22% and 85.56%, respectively (Table 3). These data, if reviewed in the context of the earlier studies, show that ProEx C staining may prove to be a valuable tool in the armamentarium for diagnosing cervical disease.[16,31] Ours, however, was a small study of 200 patients with ASC-US, and the results cannot be compared with the ALTS trial of 3488 patients with an ASC-US diagnosis and 2 years of follow-up.[8,9]

Also, based on our study, there are 2 functions for the ProEx C stain. First, it serves as a highlighter to identify atypical cells on the slide, even at a low microscopic magnification, which potentially aids in the identification of rare high-grade disease cells. Second, the stain has an interpreter function, where the presence of brown nuclear staining of atypical cells can correlate with a high likelihood of CIN 2+ disease.

ProEx C staining may occasionally be observed in benign endocervical and metaplastic cells, which was reported by Shroyer et al[31] and was noted in 92 of our cases. This finding may represent a potential diagnostic pitfall, and it highlights the importance of correlating cell morphology with ProEx C staining results. The exact etiology of why benign endocervical or metaplastic cells express this stain is not yet ascertained. A plausible explanation may be that inflammation and its mediators may be the culprits that are causing expression in these innocuous cells. However this is an important issue in the use of ProEx C staining for detecting high-grade disease because not all nuclear staining should be interpreted as a positive result. Positive nuclear staining results should be reported as positive findings only when those cells are morphologically evaluated and found to be cytologically atypical.

In conclusion, our current study suggests that ProEx C could be used to improve the diagnostic accuracy of cervical cytology in liquid-based specimens such as SurePath. This marker may also be useful in detecting underlying high-grade lesions in patients with low-grade Pap test results, which would be very useful in the clinical management of those patients. In the future, this marker may also

serve as a useful cytologic adjunct in improving the sensitivity and specificity of the Pap test.

References

1. Edwards BK, Brown ML, Wingo PA, et al. Annual report to the nation on the status of cancer, 1975–2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst.* 2005;97:1407–1427.
2. Ferlay J, Bray F, Pisani P, Parkin DM. *GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide.* Version 1.0. Lyon, France: IARC Press; 2001. *IARC Cancer Base*; no 5.
3. Stoler MH. Cervical cancer screening in the HPV era: what is the standard of care? In: Programs and abstracts of Pathology Today: American Society for Clinical Pathology 2005 Annual Meeting; October 8–11, 2005; Seattle, Wash. Session SP 20.
4. Kinney W, Sung HY, Kearney KA, Miller M, Sawaya G, Hiatt RA. Missed opportunities for cervical cancer screening of HMO members developing invasive cervical cancer. *Gynecol Oncol.* 1998;71:428–430.
5. Kinney WK, Manos MM, Hurley LB, Ransley JE. Where is the high grade cervical neoplasia?: the importance of minimally abnormal Papanicolaou diagnoses. *Obstet Gynecol.* 1998;91:973–976.
6. Sawaya GF, Grimes DA. New technologies in cervical cytology screening; a word of caution. *Obstet Gynecol.* 1999;94:307–310.
7. Nanda K, McCrory DC, Myers ER. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytological abnormalities: a systematic review. *Ann Intern Med,* 2000;132:737–743.
8. Cox JT, Schiffman M, Solomon D; for the ASCUS-LSIL Triage Study (ALTS) Group. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. *Am J Obstet Gynecol.* 2003;188:1406–1412.
9. Sherman ME, Solomon D, Schiffman M; for the ASCUS-LSIL Triage Study (ALTS) Group. Qualification of ASCUS: a comparison of equivocal LSIL and equivocal HSIL cervical cytology in the ASCUS LSIL Triage Study. *Am J Clin Pathol.* 2001;116:386–394.
10. 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.
11. Solomon D, Schiffman M, Tarone R; for the ALTS Study Group. 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.
12. Waxman AG. Guidelines for cervical cancer screening: history and scientific rationale. *Clin Obstet Gynecol.* 2005;48:77–97.
13. Wang SS, Walker H, Schiffman M, Solomon D. Evaluating the risk of cervical precancer with a combination of cytologic, virologic, and visual methods. *Cancer Epidemiol Biomarkers Prev.* 2005;14(11, pt 1):2665–2668.
14. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst.* 2003;95:46–52.
15. Schiffman M, Khan MJ, Solomon D, et al. A study of the impact of adding HPV types to cervical cancer screening and triage tests. *J Natl Cancer Inst.* 2005; 97:147–150.
16. Kelly D, Kincaid E, Fransier Z, Rosenthal DL, Clark DP. Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx C). *Cancer.* 2006;108:494–500.
17. Malinowski DP. Molecular diagnostic assays for cervical neoplasia: emerging markers for the detection of high-grade cervical disease. *Biotechniques.* 2005; 38:S17–S23.
18. Murphy N, Ring M, Heffron CCBB, et al. p16INK4a, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. *J Clin Pathol.* 2005;58:525–534.
19. Chen Y, Miller C, Mosher R, et al. Identification of cervical cancer biomarkers by cDNA and tissue microarrays. *Cancer Res.* 2003;63:1927–1935.
20. Santin AD, Zhan F, Bignotti E, et al. Gene expression profiles of primary HPV 16 and HPV 18 infected early stage cervical cancers and normal cervical epithelium: identification of novel candidate biomarkers for cervical cancer diagnosis and therapy. *Virology.* 2005;331:269–291.
21. Freeman A, Morris LS, Mills AD, et al. Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. *Clin Cancer Res.* 1999; 5:2121–2132.
22. Champoux JJ. DNA topoisomerases: structure, function, and mechanism. *Annu Rev Biochem.* 2001;70:369–413.
23. Solomon D, Nayar R, eds. *The Bethesda System for Reporting Cervical Cytology.* 2nd ed. New York, NY: Springer; 2004.
24. American Cancer Society. *Cancer Facts and Figures 2008.* Atlanta, Ga: American Cancer Society; 2008.
25. Schiffman M, Solomon D. Findings to date from the ASC-US–LSIL Triage Study (ALTS). *Arch Pathol Lab Med.* 2003;127:946–949.
26. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of HPV types associated with cervical cancer. *N Engl J Med.* 2003;348:518–527.
27. Lörincz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. *Arch Pathol Lab Med.* 2003;127:959–968.
28. Arbyn M, Buntinkx F, Van Ranst M, et al. Virologic versus cytologic triage of women with equivocal pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J Natl Cancer Inst.* 2004;96:280–293.
29. de Cremoux P, Coste J, Sastre-Garau X, et al. Efficiency of the hybrid capture 2 HPV DNA test in cervical cancer screening: a study by the French Society of Clinical Cytology. *Am J Clin Pathol.* 2003;120:492–499.
30. Guo M, Hu L, Baliga M, et al. The predictive value of p16INK4a and hybrid capture 2 human papillomavirus testing for high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol.* 2004;122:894–901.
31. Shroyer KR, Homer P, Heinz D, Singh M. Validation of a novel immunocytochemical assay for topoisomerase II-α and minichromosome maintenance protein 2 expression in cervical cytology. *Cancer.* 2006;108:324–330.