Short Communication

Evaluating the Risk of Cervical Precancer with a Combination of Cytologic, Virologic, and Visual Methods

Sophia S. Wang[1] Joan L. Walker[3] Mark Schiffman[1] Diane Solomon,[2] and for the Atypical Squamous Cell of Undetermined Significance/ Low-Grade Squamous Intraepithelial Lesion Triage Study Group

Divisions of [1]Cancer Epidemiology and Genetics and [2]Cancer Prevention, National Cancer Institute, NIH, Bethesda, Maryland and [3]Department of Obstetrics and Gynecology, University of Oklahoma, Oklahoma City, Oklahoma

Abstract

Several test modalities (cytologic, molecular, and visual) may be used for cervical cancer screening, triage, and follow-up. Although no currently available single test for cervical neoplasia can detect disease with both high sensitivity and specificity, combinations of available tests allow for improved risk prediction. We therefore evaluated the combination of liquid-based cytology (LBC), human papillomavirus (HPV) DNA testing, and visual inspection (cervicography), taken at a single point in time, to predict risk of subsequent cervical intraepithelial neoplasia 3 (CIN3) or cancer developing within 2 years in a triage population of 5,060 women referred for equivocal or mildly abnormal cytology. The concurrent administration of all three test modalities showed that combinations of these test modalities permitted clear and distinct risk stratification. Among HPV-positive women with high-grade LBC and high-grade cervicography results, 79.1 % [95% confidence interval (95% CI), 64.0- 90.0] were diagnosed with histologic CIN3 or cancer within 2 years, supporting a ''see-and-treat'' clinical application. Conversely, only 1.4% (95% CI, 0.7-2.5) of women with a negative HPV, normal cervigram, and second normal cytology result developed CIN3 or cancer. Because this low absolute risk was largely attributable to the negative HPV test, our results suggest a lack of benefit for a secondary or tertiary test result given an HPV-negative test result. Within HPV-positive women, however, we observed a steadily increasing absolute risk for cervical precancer/cancer with increasing numbers and severity of abnormal test results. We conclude that the clear discrimination of cervical cancer risk provided by multiple test modalities is consistent with our understanding of cervical etiology related to HPV natural history. (Cancer Epidemiol Biomarkers Prev 2005;14(11):2665–8)

Introduction

Cervical cancer remains the second most common female cancer worldwide (1). Of the 55 million Papanicolaou tests conducted annually in the United States, over one million will be identified as low-grade squamous intraepithelial lesions (LSIL) and approximately two million will be interpreted as atypical squamous cells of undetermined significance (ASCUS; ref. 2). New and refined test modalities, therefore, continue to be developed for cervical cancer screening and triage. Tests currently include (*a*) evaluation of cervical cells by liquid-based cytology; (*b*) DNA detection of high-risk types of human papillomavirus (HPV), the etiologic agent for cervical neoplasia; and (*c*) visual inspection of the cervix by colposcopy or related methods. Cytologic, molecular, and visual methods may all play a role in cervical cancer screening or triage in the future (3, 4).

Cervical cytology has been the mainstay of cervical cancer screening for >50 years. Recently, however, HPV testing has been incorporated into screening guidelines (5); current recommendations include the option of dual cytology and HPV testing in women ages ≥30 years (5). For triage, HPV testing is now preferred to clarify an ASCUS cytology result (6). Cervicography, cervical probe devices, and colposcopy provide a visual assessment of the cervix and are used for triage and diagnostic purposes.

In the present study, independent and combined test results for the three test modalities conducted concurrently at enrollment in the ASCUS/LSIL Triage Study (ALTS) were evaluated for their absolute risk values (positive predictive value) for women developing subsequent cervical intraepithelial neoplasia grade 3 (CIN3) or cancer during the study's 2-year follow-up period. Our goal was to determine whether the combination of the three possibly complementary methods (3) would clearly show increased accuracy in predicting cervical precancer and cancer in a triage population.

Materials and Methods

Study Population. We analyzed data from the ALTS, a multicenter, randomized trial described previously (7, 8). Briefly, women were referred after a community conventional cytology was interpreted as ASCUS (n = 3488) or LSIL (n = 1572). Participants were enrolled from November 1996 through December 1998 at four clinical sites (University of Alabama, Birmingham, AL; Magee-Women's Hospital of the University of Pittsburgh Medical Center Health System, Pittsburgh, PA; Oklahoma University Health Sciences Center, Oklahoma City, OK; and University of Washington, Seattle, WA), on average 2 months following their referral cytology. The median age of women at enrollment was 25 years. At study enrollment, the following three test modalities were administered: a second (repeat) cytology using liquid-based cytology (LBC), an HPV test, and a visual examination of the cervix. Women were

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Requests for reprints: Sophia S. Wang, Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 6120 Executive Boulevard, EPS MSC# 7234, Bethesda, MD 20892-7234. Phone: 301-402-5374; Fax: 301-402-0916. E-mail: wangso@mail.nih.gov

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Table 1. Risks (positive predictive values) for single, two-stage, and three-stage strategies for CIN3$^+$ as diagnosed by the ALTS Pathology QC Group among women referred for ASCUS or LSIL cytology

LBC		HPV test					
		Negative			Positive		
No. CIN2$^+$/ total	Risk, % (95% CI)	No. CIN2$^+$/ total 66/1796		Risk, % (95% CI) 3.7 (2.9-4.7)	No. CIN2$^+$/ total 829/3023		Risk, % (95% CI) 27.4% (25.8-29.1)
Normal, 116/1754	6.6 (5.5-7.9)	34/1054		3.2% (2.2-4.5)	76/620		12.3% (9.8-15.1)
		Visual assessment	No. CIN2$^+$/total	Risk (95% CI)	Visual assessment	No. CIN2$^+$/total	Risk (95% CI)
		Normal	17/772	2.2 (1.3-3.5)	Normal	28/388	7.2 (4.8-10.3)
		Atypical	12/108	11.1 (5.9-18.6)	Atypical	14/95	14.7 (8.3-23.5)
		Low grade	5/133	3.8 (1.2-8.6)	Low grade	33/116	28.4 (20.5-37.6)
		High grade/cancer	0/7	0 (0.0-41.0)	High grade/cancer	1/7	14.3 (0.4-57.9)
ASCUS, 197/1495	13.2 (11.5-15.0)	23/620		3.7% (2.4-5.5)	170/822		20.7% (18.0-23.6)
		Visual assessment	No. CIN2$^+$/total	Risk, % (95% CI)	Visual assessment	No. CIN2$^+$/total	Risk, % (95% CI)
		Normal	11/412	2.7 (1.3-4.7)	Normal	62/451	13.7 (10.7-17.3)
		Atypical	3/79	3.8 (0.8-10.7)	Atypical	30/134	22.4 (15.6-30.4)
		Low grade	8/102	7.8 (3.4-14.9)	Low grade	59/200	29.5 (23.3-36.3)
		High grade/cancer	0/1	0 (0.0-97.5)	High grade/cancer	16/23	69.6 (47.1-86.8)
LSIL, 305/1342	22.7 (20.5-25.1)	6/103		5.8% (2.2-12.2)	287/1160		24.7% (22.3-27.3)
		Visual assessment	No. CIN2$^+$/total	Risk, % (95% CI)	Visual assessment	No. CIN2$^+$/total	Risk, % (95% CI)
		Normal	2/63	3.2 (0.4-11.0)	Normal	89/500	17.8 (14.5-21.4)
		Atypical	1/10	10.0 (0.3-44.5)	Atypical	62/241	25.7 (20.3-31.7)
		Low grade	2/26	7.7 (0.9-25.1)	Low grade	114/361	31.6 (26.8-36.6)
		High grade/cancer	1/1	100.0 (2.5-100)	High grade/cancer	16/33	48.5 (30.8-66.5)
HSIL$^+$, 312/445	70.1 (65.6-74.3)	3/9		33.3% (7.5-70.1)	294/412		71.4% (66.7-75.7)
		Visual assessment	No. CIN2$^+$/total	Risk, % (95% CI)	Visual assessment	No. CIN2$^+$/total	Risk, % (95% CI)
		Normal	3/7	42.9 (9.9-81.6)	Normal	67/117	57.3 (47.8-66.4)
		Atypical	0/1	0 (0.0-97.5)	Atypical	37/65	56.9 (44.0-69.2)
		Low grade	0/0		Low grade	146/176	83.0 (76.6-88.2)
		High grade/cancer	0/0		High grade/cancer	39/43	90.7 (77.9-97.4)

NOTE: Shaded areas, single-strategy risk estimates (24 missing values for LBC, 24 missing values for HPV test); cross-hatched area, dual-strategy risk estimates (cytology and HPV; 260 missing values); white boxes, combination of three strategies (cytology, HPV, and cervicography; 388 missing values).

invited for follow-up visits every 6 months with a final exit colposcopy visit conducted after 2 years to promote complete detection of disease outcomes. Written informed consent was obtained from each subject; the study was conducted with the approval of local institutional review boards and in accordance with the National Cancer Institute Institutional Review Board.

LBC. Upon referral into ALTS, a ThinPrep (Cytyc Corp., Boxborough, MA) cytology specimen was collected and prepared as previously described (7), on all women at enrollment; LBC interpretations from each clinical center were categorized according to the 1991 Bethesda System as normal, ASCUS, LSIL, or high-grade squamous intraepithelial lesion or greater (e.g., HSIL and cancer). These interpretations were made independently from HPV and cervicography results.

HPV DNA Testing. HPV testing was conducted using the hybrid capture 2 assay (Digene Corp., Gaithersburg, MD) for 13 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) using residual PreservCyt (Cytyc) cytology aliquots collected at enrollment (9).

Visual Examination. At enrollment, high-resolution cervical photographs were taken after application of acetic acid (Cervicography, National Testing Laboratories Worldwide, Fenton, MO). The cervigrams were interpreted by expert reviewers at the National Testing Laboratories without any additional clinical information. We had enrollment colposcopy impressions from 1,836 women randomized to the immediate colposcopy arm of the trial; in this group of women, the agreement between cervigram and colposcopy impressions (normal, atypical/low grade, high grade/cancer) was fair (percent agreement = 48%). Recognizing that the reproducibility of all visual assessment is not high even among experts (10), we used the cervicography data in lieu of colposcopy impression, because the results were available for all women and were rendered by separate reviewers without clinical information, such as HPV or LBC test results. We grouped cervigram interpretations as normal, atypical (A or P_0), low grade (P_1), or high grade/cancer (P_2/P_3; ref. 11).

Pathology Outcome. All women were followed every 6 months for 2 years regardless of randomization arm and treated for a clinical center diagnosis of histologic CIN2 or worse (e.g., CIN2, CIN3, and cancer). In the current analysis, we therefore included CIN2 as an intermediate end point of clinical interest, as detected by the clinical center. Our main histologic end point of interest, however, was the more stringent surrogate end point for cancer risk, defined as precancer [i.e., CIN3, or cancer (CIN3$^+$)] as diagnosed during the 2-year follow-up by an expert pathology review group (9, 12). An independent expert pathology review panel masked to other test results was used to identify and confirm all CIN3$^+$ diagnoses to improve the diagnostic accuracy.

Statistical Analyses. We evaluated the absolute risks for each independent test modality, and for their combinations, 'for the histologic diagnosis of CIN3$^+$ (n = 542), in the 2 years of follow-up in ALTS. We included parallel analyses for histologic diagnosis of CIN2$^+$ (n = 932) diagnosed at the clinical centers for those interested in this treatment threshold. For each of the three test modalities (independently, in pairwise, and three-way combinations), we calculated the absolute risks or positive predictive value, defined as the percentage of women diagnosed with the disease end point given a specific positive test result or combination of results;

respective 95% confidence intervals (95% CI) were also calculated. In addition to the overall analyses, we also conducted analyses stratified by referral status (LSIL versus ASCUS) and by age (<30 and >30 years). All analyses were conducted using SAS version 8.2 (SAS Institute, Inc., Cary, NC).

Results

As shown in Table 1, despite the referral cytologic abnormality, the overall 2-year risk of CIN3$^+$ among HPV-negative women was only 1.8% (95% CI, 1.3-2.6) and was 1.4% (95% CI, 0.7-2.5) among women with all three test results of negative. However, the low absolute risk was largely due the HPV-negative test result, and in general, HPV-negative test results predicted low risks for CIN3$^+$ regardless of cervicography and LBC results.

On the other extreme, the overall risk among women with an HSIL or greater LBC interpretation was 43.8% (95% CI, 39.2-48.6) in all women and 44.4% (95% CI, 39.6-49.4) among HPV-positive women. The addition of cervicography results further stratified this risk, first lowering it to 27.4% (95% CI, 19.5-36.4) and 27.7% (95% CI, 17.3-40.2) for normal and atypical cervicography results, respectively, but increasing risk to 53.4% for low-grade cervicography results. The highest risk of 79.1% (95% CI, 64.0-90.0) was observed among HPV-positive women with HSIL cytology and a visual impression of high grade/cancer thus clearly showing the added value of each test result. We further note that because there were no HPV-negative women identified with a HSIL or greater LBC result and high grade/cancer cervicography result, the absolute risk was virtually identical for the dual-test strategy based on LBC and cervicography results alone. Overall, the addition of each test modality improved the predictive value for CIN3$^+$ outcome, particularly for women with HPV-positive test results.

As expected from the more liberal threshold for disease outcome, the risks for clinical center diagnoses of CIN2$^+$ were higher than for Pathology QC diagnoses of CIN3$^+$ (Table 2); 90.7% of HPV-positive women with HSIL and visual impressions of high grade/cancer were diagnosed with CIN2$^+$ during the 2 years of follow-up in ALTS. As was observed for CIN3$^+$ outcomes, the addition of each test modality improved the overall predictive values for CIN2$^+$ outcomes, particularly for women with HPV-positive test results.

Discussion

In our ALTS triage population, we show that the accuracy in predicting cervical precancer and cancer developing within 2 years is now very high, given the availability of three complementary viral, cytologic, and visual test results measured concurrently. It is clear that the accuracy of the combined test results surpasses that of individual or even dual test strategies, for both CIN2 and CIN3 or greater outcomes.

Strengths of our current analysis included independent test result assessment; cervicography interpretations were made independent from HPV and LBC test results and even from colposcopy results. Another strength of the ALTS is in the number of HPV-positive women, which although much higher in our triage population than that observed for screening populations, allowed for the present risk stratification within HPV-positive women using cytology and cervicography test

Table 2. Risks (positive predictive values) for single, two-stage, and three-stage strategies for CIN2$^+$ as diagnosed by the ALTS clinical center pathologists among women referred for ASCUS and LSIL cytology

LBC		HPV test					
		Negative			Positive		
No. CIN3+ /total	Risk, % (95% CI)	No. CIN3$^+$/total		Risk, % (95% CI)	No. CIN3$^+$/total		Risk, % (95% CI)
		33/1796		1.8% (1.3-2.6)	486/3023		16.1% (214.8-17.4)
Normal, 73/1754	4.2 (3.3-5.2)	19/1054		1.8% (1.1-2.8)	50/620		8.1% (6.0-10.5)
		Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)	Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)
		Normal	11/772	1.4 (0.7-2.5)	Normal	15/388	3.9 (2.2-6.3)
		Atypical	4/108	3.7 (1.0-9.2)	Atypical	5/95	5.3 (1.7-11.9)
		Low grade	4/133	3.0 (0.8-7.5)	Low grade	29/116	25.0 (17.4-33.9)
		High grade/cancer	0/7	0 (0.0-41.0)	High grade/cancer	1/7	14.3 (0.4-57.9)
ASCUS, 113/1495	7.6 (6.3-9.0)	7/620		1.1% (0.5-2.3)	104/822		12.7% (10.5-15.1)
		Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)	Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)
		Normal	4/412	1.0 (0.3-2.5)	Normal	37/451	8.2 (5.8-11.1)
		Atypical	0/79	0 (0.0-4.6)	Atypical	22/134	16.4 (10.6-23.8)
		Low grade	3/102	2.9 (0.6-8.4)	Low grade	30/200	15.0 (10.4-20.7)
		High grade/cancer	0/1	0 (0.0-97.5)	High grade/cancer	12/23	52.2 (30.6-73.2)
LSIL, 160/1342	11.9 (10.2-13.8)	5/103		4.9% (1.6-11.0)	148/1160		12.8% (10.9-14.8)
		Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)	Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)
		Normal	2/63	3.2 (0.4-11.0)	Normal	42/500	8.4 (6.1-11.2)
		Atypical	1/10	10.0 (0.3-44.5)	Atypical	25/241	10.4 (6.8-14.9)
		Low grade	1/26	3.8 (0.1-19.6)	Low grade	69/361	19.1 (15.2-23.6)
		High grade/cancer	1/1	100 (2.5-100.0)	High grade/cancer	9/33	27.3 (13.3-45.5)
HSIL$^+$, 195/445	43.8 (39.2-48.6)	2/9		22.2% (2.8-60.0)	183/412		44.4% (39.6-49.4)
		Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)	Visual Assessment	No. CIN3$^+$/total	Risk, % (95% CI)
		Normal	2/7	28.6 (3.7-71.0)	Normal	32/117	27.4 (19.5-36.4)
		Atypical	0/1	0 (0.0-97.5)	Atypical	18/65	27.7 (17.3-40.2)
		Low grade	0/0		Low grade	94/176	53.4 (45.8-60.9)
		High grade/cancer	0/0		High grade/cancer	34/43	79.1 (64.0-90.0)

NOTE: Shaded areas, single-strategy risk estimates (24 missing values for LBC, 24 missing values for HPV test); cross-hatched area, dual-strategy risk estimates (cytology and HPV; 260 missing values); white boxes, combination of three strategies (cytology, HPV, and cervicography; 388 missing values).

results. Some strata included very few women, such as HPV-negative women with LSIL and HSIL$^+$ LBC test results; the low number of women in these strata in part shows and further supports the minimal value of additional cytology or visual tests in HPV-negative women. In our analyses, we also evaluated risks stratified by referral status (ASCUS and LSIL); because of the lack of difference in the risk estimates, we present combined results. We also evaluated risks stratified by age (<30 and >30 years), but in the absence of informative differences, we combined the results for all women.

Although various independent test strategies have been previously evaluated (9, 13, 14), we report here our evaluation of concurrent test strategies in identifying women at highest risk for cervical neoplasia. The ALTS study provided a unique and valuable resource for evaluating the accuracy of concurrent test strategies; specifically, it allowed us to evaluate the current status of knowledge regarding the natural history of cervical neoplasia and the accuracy of complementary test strategies currently available. We note that the concurrent administration of all three tests is not standard protocol for clinical practice nor do we suggest it; it would be an inefficient use of resources and further lead to a large number of women with multiple equivocal or low-grade test results. Efficient and cost-effect triage strategies that take into account the timing of the test, the order and number of tests necessary, and the specific populations tested (e.g., by age) for these women are thus needed (15).

The present results are generalizable to a triage population of U.S. women referred for LSIL or ASCUS cytology who have had HPV testing by hybrid capture 2 and visual evaluation of the cervix (e.g., cervicography or colposcopy). These data are not representative of the screening population at large; risk estimates reported are higher than would be expected in the general population, most of whom will not have had an equivocal or mildly abnormal Papanicolaou test result, as was indicated for enrollment in the present ALTS protocol. Nevertheless, we do believe our results for each of the three complementary tests observed for this triage population would likely be similar for a screening population. As would likely be indicated for screening, our triage results support that within HPV-negative women, further testing is not likely needed (although continued follow-up and screening visits should continue according to current guidelines).

In conclusion, these data extend previous ALTS findings, which compare the performance of single-test modalities (16, 17). Current triage and management strategies yield combinations of test results that, taken together, provide increased discrimination of risk compared with any single test. We believe this is consistent with our present and clear understanding of the natural history of cervical cancer. Although we clearly show that the combinations of test modalities can pinpoint a small group at extremely high risk of precancer, the administration of all three tests simultaneously is inefficient for triage and screening settings and we thus continue to search for biomarkers of cancer risk that can be measured as a single test, with high reliability and accuracy.

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