

Comparison of Human Papilloma Virus Testing and Spectroscopy Combined With Cervical Cytology for the Detection of High-grade Cervical Neoplasia

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■ Abstract

Objective. This study compared the performance of cervical cytology plus human papilloma virus testing (Pap + HPV) or cervical spectroscopy (Pap + CS) for identifying high-grade cervical neoplasia in a high-risk population of women referred for colposcopy.

Materials and Methods. Each of 113 subjects underwent spectroscopy, thin-layer cytology, HPV testing, colposcopy, biopsy when indicated, and/or endocervical curettage. Evaluable data for analysis were collected for 102 of the subjects. Sensitivity and specificity were calculated for both strategies.

Results. Pap + HPV and Pap + CS achieved equivalent sensitivities (95%) for high-grade lesions, with both detecting 17 of 18 histology confirmed cervical intraepithelial neoplasia (CIN) 2+ lesions. Pap + HPV had a specificity of only 27.4% compared with 65.5% for Pap + CS ($p < .0001$).

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Conclusions. Spectroscopic interrogation of the cervix is equally sensitive and 2-fold more specific than HPV testing when combined with cervical cytology for identifying high-grade cervical neoplasia. ■

Key Words: spectroscopy, HPV testing, cervical neoplasia

Under current clinical practice guidelines, to find the few women who actually do harbor high-grade cervical neoplasia, many more women are evaluated with additional Pap tests, human papilloma virus (HPV) testing, colposcopy and biopsy.

Evidence-based guidelines for the management of abnormal cervical cytologies, based in part on recent clinical studies such as the ASCUS-LSIL Triage Study [1–6], rely heavily upon testing for HPV, known to be necessary for the development of cervical neoplasia [7]. However, HPV testing is an indirect marker of neoplasia, and the positive predictive value of a positive HPV test is approximately 16% [8] because of its high prevalence in the general population, especially in women with abnormal Pap tests [9] and in younger women [5].

More direct and accurate indicators of neoplasia would have the advantage of focusing attention and

resources on those cytological abnormalities that truly represent disease. A potential strategy to fill this need is spectroscopy, a noninvasive light-based technology capable of providing immediate and objective results in the detection of neoplasia.

This study compared the performance of quantitative optical spectroscopy to HPV testing when both are combined with cervical cytology for identifying women with high-grade cervical intraepithelial neoplasia (CIN 2+).

MATERIALS AND METHODS

The study is a prospective, double-masked, single-arm trial, whereby each subject served as their own control. Women previously referred for colposcopy, in most cases in response to an abnormal cervical cytology, were offered participation in the study. Inclusion criteria were age 16 years and older, a Pap test within 120 days before study participation, and scheduled for colposcopy for standard clinical indications. Exclusion criteria included pregnancy, previous radiation therapy of the urogenital tract, or current menstruation. Recruitment of subjects took place from April 2003 to January 2004. This study was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas.

Device

The cervical spectroscopy (CS) device used in this study (Guided Therapeutics, Inc., Norcross, GA) is a non-significant risk device by Food and Drug Administration standards that noninvasively collects and analyzes fluorescence and reflectance spectra from the cervix. No contrast agents, such as acetic acid, were applied to the cervix before taking CS measurements. The CS device interrogated a plurality of equally spaced points over a 1-in-diameter area of the cervix during a 4-minute period using a xenon arc lamp as an illumination source. Fluorescence measurements used band-filtered light from the xenon arc lamp within the 300- to 500-nm range. Each of the fluorescence wavelengths were applied automatically under software control in a predetermined order and scan pattern. For cervical tissue reflectance measurements, broadband spectral output ranging from approximately 350 to 900 nm was applied during the scan. All light exposures were filtered to ensure all ultraviolet energy below 295 nm was adequately blocked. The light was delivered and recollected from the cervix via an optical system that terminated with a handheld device. A hollow tube was

connected to the handheld device and inserted through a speculum into the vagina with the distal end placed against the cervix. A separate imaging channel allowed real-time video imaging of the cervix for positioning guidance and static imaging to document cervical position and contact tube placement. The resultant fluorescent and reflected light from the cervical tissue of each subject was digitized and stored for further processing and analysis. Spectroscopy data were unavailable to the examining clinician at the time of subject evaluation and, therefore, could not be used to guide colposcopy or direct biopsy.

Cervical Spectroscopy Diagnostic Algorithm

The algorithm used to generate the spectroscopic indices for this study was developed using diagnostically informative fluorescent and reflectant wavelengths as determined by previous studies with more than 700 subjects enrolled in multicenter trials conducted in the United States from 1999 to 2002. These previous studies were used to determine the optimal wavelengths and coefficients that best distinguished high-grade neoplasia from benign conditions.

Procedure

Informed consent was obtained before examination. For each subject, spectroscopic interrogation of the cervix was completed, followed by cervical sampling for liquid-based thin-layer cytology (ThinPrep; Cytoc Corp., Boxborough MA) and HPV DNA testing for the presence of any of the 13 high-risk HPV types Hybrid Capture II; Digene Corp., Gaithersburg, MD. Colposcopic evaluation was then performed next by 1 of 2 experienced colposcopists (C.W. or W.G.). Acetic acid 5% was applied to the cervix, after which a digital photograph was taken to document any colposcopically visible lesions and the location of any clinically indicated biopsies performed. If no lesions were evident with acetic acid, Lugol's iodine solution was applied to the cervix to identify any additional areas of potential abnormality (iodine stain negative) for biopsy. Endocervical curettage (ECC) was performed at the conclusion of all colposcopic examinations. Investigators and subjects were masked to all spectroscopic data collected.

Histopathology Quality Control

Tissue biopsies were fixed and processed for histopathology evaluation per standard clinical practice. An additional slide adjacent to the diagnostic slide was

prepared and sent for independent evaluation by contributing author E.W., a specialist in gynecologic pathology. If E.W. disagreed with the diagnosis of the clinical site pathologist (R.A.), the slide was evaluated by a third expert pathologist (S.R.). A case was assigned to a diagnostic category when either the site pathologist and E.W. agreed or 2 of 3 diagnoses agreed as to the histological diagnosis. Pathology diagnoses were categorized as benign, CIN 1, or CIN 2+, using the most severe disease grade for each case. A case was considered not evaluable when all 3 pathologists disagreed. The final quality controlled pathology decision was used as the gold standard by which sensitivity and specificity of the 2 strategies were calculated.

Statistical Analysis and Rules for Assigning a Subject as Positive or Negative for CIN 2+

The following rules determined whether each case of histology-confirmed CIN 2+ was detected by either strategy.

The Pap + HPV strategy was considered to have detected CIN 2+ if any of the following conditions were met:

- day-of-study Pap result low- or high-grade squamous intraepithelial lesion (LSIL or HSIL) or ASC-H, regardless of HPV status;
- day-of-study Pap result ASC-US and HPV test positive;
- day-of-study HPV status was not available (inadequate specimen), referral HPV test result positive;
- day-of-study Pap negative, referral and day-of-study HPV tests both positive (two successive HPV positives);
- for item (d), if referral HPV is unavailable, referral Pap must be LSIL or HSIL.

Pap + CS performance was based upon the spectroscopic index calculated for each subject by the CS device software based upon the collected spectral data. The Pap + CS strategy was considered to have detected CIN 2+ if any of the following criteria were met:

- spectroscopy index > 1.5, if Pap is LSIL or HSIL;
- spectroscopy index > 2.0, if Pap is ASC-US;
- spectroscopy index > 2.5, if Pap is benign or normal.

McNemar test was used to statistically compare the sensitivities and specificities of the Pap + HPV and Pap + CS strategies.

RESULTS

Of the 113 subjects who consented to participate, 109 qualified and completed the study. The other 4 were ineligible by inclusion criteria or withdrew from the

study after the consent process had been completed. Data were not collected from 5 subjects because of device malfunction ($n = 3$) or operator error ($n = 2$). The median age of the remaining 104 study subjects was 31 years (range, 16–57 years), with 50 subjects (48%) younger than 30 years. There were 59 African American (57%), 33 Hispanic (31%), 11 white (11%), and 1 Asian American (1%) subjects. Two study subjects' data were not included in the data analysis because of 3-way histopathology discordances pertaining to their histological diagnoses. Two cases with borderline histology (e.g., CIN 1–2) were included in the analysis. Data from 102 study subjects were used for analysis.

The protocol called for all subjects to have both a referral Pap test within 120 days of the study as well as a Pap test on day of the study. Table 1 compares the results of these 2 Pap tests. Eighty-nine percent of referral Pap tests were either ASC-US or LSIL. Seven (7%) were referred for HSIL or ASC-H Pap tests. Four (4%) subjects had negative referral Pap tests and underwent colposcopy due to gross cervical lesions or history of an abnormal Pap test. Consistent with the known lack of reproducibility of Pap results, only 66 (67%) of the 98 patients referred for abnormal Pap tests had abnormal Pap tests on the day of study. Of note, 3 (43%) of the 7 referred for HSIL Pap tests had normal cytologies on day of study. Five day-of-study cytologies were insufficient for evaluation.

Although HPV testing at the time of the referral Pap was not required for inclusion in the study, there were 70 subjects who did have comparison sets of HPV data (Table 2). Forty-one (58.6%) tested positive on both HPV tests. The rate of insufficient samples for HPV testing on the day of study was 12 (11.8%) of 102. Of the 59 subjects with HPV tests performed on both referral and day of study, only 3 (5%) were negative for high-risk HPV on both, with 95% positive on one or both HPV tests.

Table 1. Comparison of Referral Pap Test and Day-of-study Pap Test

Referral Pap test	Day-of-study Pap test					Total
	Negative	ASC-US	LSIL	HSIL or ASC-H	Insufficient sample	
Negative	1	1	1	0	1	4
ASC-US	16	18	15	3	4	56
LSIL	13	5	14	3	0	35
HSIL or ASC-H	3	0	1	3	0	7
Total	33	24	31	9	5	102

Table 2. Comparison of Referral HPV Test and Day-of-study HPV Test

Referral HPV test	Day-of-study HPV test			
	Negative	Positive	Insufficient sample	Total
Negative	3	1	1	5
Positive	14	41	10	65
Not performed or unavailable	6	25	1	32
Total	23	67	12	102

Referral and day-of-study Pap test results are compared with their associated HPV test results in Tables 3 and 4. Consistent with current standard clinical practice, most of the referral HPV tests (55 of 70) were performed in response to ASC-US Pap results. Likewise, the majority of referral LSIL, HSIL, or ASC-H cytologies had not undergone reflex HPV testing.

If HPV testing was performed on the day-of-study, positive results were obtained for 39.3% (11 of 28) of benign Pap tests, 81.2% (18 of 22) of ASC-US Pap tests, 93.5% (29 of 31) of LSIL Pap tests, and 100% (9 of 9) of HSIL or ASC-H Pap tests. Overall, 65 (64%) of the 102 study patients had positive referral HPV tests, and 67 (66%) had positive day-of-study HPV testing.

Sensitivity and Specificity of Pap + HPV versus Pap + CS

Of the 102 patients analyzed, 18 cases (18%) of histological high-grade cervical neoplasia (CIN 2+) were diagnosed. There were no invasive cancers. There were 2 intermediate cases with histopathology diagnosed as CIN 1 to 2. In one case, the lesion was ectocervical; in the other, the CIN was evident in the ECC.

Both diagnostic strategies correctly diagnosed all 18 unequivocal CIN 2+ lesions (sensitivity = 100%). Of the 2 intermediate (CIN 1–2) lesions, the ectocervical lesion was not detected by either detection strategy. The case of CIN 1 to 2 diagnosed by ECC alone was detected by both strategies. Counting the borderline cases as high-grade, both strategies demonstrated an equal sensitivity of 95%.

Table 3. Referral Pap and Referral HPV Results

Referral HPV result	Referral Pap result				Total
	Benign	ASC-US	LSIL	HSIL or ASC-H	
Positive	1	55	8	1	65
Negative	0	1	3	1	5
Not performed or unavailable	3	0	24	5	32
Total	4	56	35	7	102

Table 4. Day-of-study Pap and HPV Results

Day-of-study HPV result	Day-of-study Pap result					Total
	Benign	ASC-US	LSIL	HSIL or ASC-H	Insufficient sample	
Positive	11	18	29	9	0	67
Negative	17	4	2	0	0	23
Insufficient sample	5	2	0	0	5	12
Total	33	24	31	9	5	102

Specificity for Pap + HPV was 27.4% (23 of 84), whereas for Pap + CS, specificity was 65.5% (55 of 84). This difference is statistically significant ($p < 0.0001$).

DISCUSSION

This study compared the clinical performance of cervical cytology plus either CS or HPV testing for identifying women with high-grade cervical neoplasia. Another potential use of this technology, the ability to locate cervical lesions, was not addressed in this study and is the subject of further research. Rather, this study addressed whether CS has the potential to objectively triage women to colposcopy and biopsy, because most women referred to colposcopy and biopsy do not have significant disease. For this reason, subjects included women referred for colposcopy, mostly because of abnormal cervical cytology. Although not intended to be representative of a screening population, this high-risk urban group of subjects offered a study population with an increased prevalence (18%) of high-grade neoplasia (CIN 2+).

The spectroscopy device evaluated in this study used specific light wavelengths to spatially sample the entire ectocervix and distal endocervical canal by full-depth penetration of the cervical epithelium. In contrast, cytology and colposcopy assess only the epithelial surface and are known to have significantly high false-negative rates [10–15].

Both fluorescence and reflectance spectroscopy have previously been shown to be effective in cancer diagnosis [16]. Clinical studies have characterized the performance of either fluorescence or reflectance spectroscopy in discriminating between normal tissue and different grades of epithelial cancer at several tissue sites including the cervix [17–21], colon [22–25], gastrointestinal tract [26], and skin [27]. Fluorescence measures biochemical changes that occur in the course of neoplastic transformation. The natural fluorophores present in tissue are the aromatic amino acids tyrosine,

phenylalanine, and tryptophan; the metabolites NAD(H) and FAD; and the structural proteins collagen and elastin. Fluorescence from these molecules depends upon their physiochemical environment, which includes pH, solvation, and oxidation states. Reflectance measures morphological changes associated with cancer progression by detecting changes in cell nuclei, cell size, cell appearance, and cell arrangement. Additionally, neoangiogenesis impacts the spectroscopic character of tissue. Both biochemical and morphological changes vary with the degree of neoplastic severity. Combination of the 2 spectroscopic modes is thought to improve accuracy by increasing sensitivity and correcting for interfering biochemical alterations.

In our study, both CS and HPV testing detected the presence of high-grade cervical neoplasia with sensitivity equal to 95% for CIN 2+ and 100% for CIN 3 when combined with cervical cytology. The same level of sensitivity was also found for CS without cytology but with loss of specificity. All unequivocal cases of high-grade neoplasia by histology were detected by Pap + CS and Pap + HPV strategies as well as by CS alone. There were 2 cases of borderline HSIL biopsies read as CIN 1 to 2. One of these cases was diagnosed by ectocervical biopsy and the other via ECC. The former case was not identified by PAP + CS or PAP + HPV, whereas the latter was identified by both strategies. The “missed” ectocervical CIN 1 to 2 lesions, being spectroscopy and HPV negative, brings to question whether there are cases of equivocal high-grade lesions that are not true physiological precursors of cancer.

Of clinical interest, Pap + CS demonstrated a significantly higher specificity than Pap + HPV (65.5% vs 27.4%, $p < 0.0001$). This difference in specificity could be explained by the fact that HPV testing detects a known cofactor associated with cervical neoplasia that is also commonly present in the lower genital tracts of women who do not have neoplasia. Instead, CS directly detects the cellular metabolic and structural changes that occur specifically in the presence of high-grade cervical neoplasia rather than an indirect marker of elevated risk.

CS failed to produce data in only 5 cases (4.6%); 3 failures were because of device malfunction and 2 from operator errors. This compares favorably to other screening modalities used. Cytology could not be interpreted for 6 subjects (5.5%), and HPV testing did not yield results for 12 subjects (11.0%). There were no adverse events associated with the use of CS, and subjects tolerated the procedure well as reported in previous evaluations of this technology [28].

The results are consistent with findings from previous studies [29–31] showing that the CS device is capable of detecting more than 95% of CIN 2+ with a corresponding specificity for benign cervixes of 55% in a population of women scheduled for colposcopy. The same algorithm used for distinguishing CIN 2+ disease from benign cases classified approximately 75% of CIN 1 cases as positive. However, developing a secondary algorithm could separate out 94% of the CIN 3+ and 85% of the CIN 2 lesions from approximately half of the CIN 1 lesions. When applied to our population having a 20% prevalence of CIN 2+ disease, the negative predictive value of Pap + CS would be approximately 98%. For CIN 3+ lesions with a prevalence of 10%, the negative predictive value would be approximately 99%.

Given the clinical issues regarding current standard of practice described above, a more direct approach to identifying patients with significant cervical disease is needed in the interest of reducing health costs, diagnosis delays, and patient anxiety. Screening and surveillance strategies using cytology and HPV testing alone or in combination rely on tests that are not reproducible in subjects during short time intervals [32–34]. In this study, only 65 (65%) of 98 patients referred for abnormal Pap tests had abnormal Pap tests on day of study. Of note, 3 of 7 patients referred for HSIL Pap tests had normal repeat cytologies. Furthermore, of the 41 subjects with positive referral HPV tests, 14 (34%) were negative on the day of the study. There is a need for point-of-care testing that identifies significant cervical disease and enhances effective colposcopy triage strategies.

Although the current study has investigated CS for the evaluation of women mostly with abnormal cervical cytologies, future research should evaluate its usefulness for primary screening and as an adjunct during colposcopy. Colposcopy has been considered the gold standard for the detection of cervical neoplasia for the past 30 years, but recent studies have refuted its accuracy. The diagnostic accuracy of colposcopy with directed biopsy is highly dependent on practitioner skill and experience. The sensitivity of colposcopy for the detection of high-grade cervical neoplasia has recently been reported in the range of 50% to 85% [17–20]. This has serious implications for the validation of effective biomarkers and technologies when high-grade disease is present but not identified by colposcopy, producing the appearance of false-positive results. Of equal concern, the specificity of colposcopy is reported to be approximately 50% where biopsied lesions actually represent

normal epithelium, and only about a quarter of all cervical biopsies result in a histological diagnosis of CIN 2+. In our study, prevalence of high-grade disease in biopsied cases was only 18%, similar to the 26% found for ASC-US and LSIL cases in the ASCUS-LSIL Triage Study [2,4]. Our study included women with HSIL Pap tests, which should have increased the likelihood of CIN 2+ histological findings. This conundrum has been exacerbated during the last decade by changes in cytological classification (e.g., creation of the Bethesda System's ASC-US category), thin-layer cytology, and HPV testing. Although these entities help increase the detection of high-grade dysplasia, they can also increase the number of false-positive biopsies. Colposcopic and histological overreads lead to inflated diagnoses of cervical neoplasia, resulting in unnecessary morbidity and expense.

Cervical spectroscopy is a novel, evolving technology that offers a rapid, easy-to-perform, and well-tolerated point-of-care assessment of the uterine cervix for the presence of high-grade neoplasia. This study supports earlier reports demonstrating spectroscopy's high sensitivity and superior specificity capabilities along with the added clinical convenience of immediate results. As guidelines for primary screening, triage of abnormal cytology, and postcolposcopy or treatment surveillance continue to evolve, the potential role of spectroscopy should be considered and fully investigated.

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