Effective cervical neoplasia detection with a novel optical detection system: A randomized trial

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Received 15 February 2006

Abstract

Objective. To assess whether the use of a novel optical detection system (ODS) as an adjunct to colposcopy increases the detection of biopsy-confirmed CIN 2,3.

Methods. This is a multicenter two-arm randomized trial comparing colposcopy alone with colposcopy plus a pre-commercial ODS system that utilizes fluorescence, white light tissue reflectance, and cervical video imaging. Patients were recruited from 13 colposcopy clinics in a variety of practice settings. 2299 women referred for the evaluation of an abnormal cervical cytology were randomized with stratification by cytology; subsequently 113 women were excluded for a variety of reasons. The main study outcomes were differences in true-positive rates (CIN 2,3 and cancer identified) and false-positive rates between the study arms.

Results. The true-positive (TP) rates were 14.4% vs. 11.4% (p = 0.035, one-sided) for the combined colposcopy and ODS arm compared to colposcopy-only arm, respectively, in women with either an atypical squamous cell (ASC) or low-grade squamous intraepithelial lesion (LSIL) cytology result. TP rates were similar between the two arms among women referred for the evaluation of HSIL. The 26.5% gain in true-positives observed with the use of ODS and colposcopy among women referred for an ASC or LSIL cytology was achieved with only a fractional increase in number of biopsies obtained per patient (0.30) and a modest increase in false-positive rate (4%). In the combined colposcopy and ODS arm among women with ASC or LSIL, the PPV of biopsies indicated by ODS was 15.0% and the PPV of biopsies indicated by colposcopy was 15.2%. Joint hypothesis testing indicates that ODS and colposcopy provides benefit compared to colposcopy alone among women with ASC or LSIL.

Conclusions. Combining ODS with colposcopy provides a clinically meaningful increase in the detection of CIN 2,3 in women referred for the evaluation of mildly abnormal cytology results.

Keywords: Cervical intraepithelial neoplasia; Optical spectroscopy; Colposcopy

Introduction

For the last three decades, colposcopic examination with cervical biopsy has been considered the standard of care for evaluating women with abnormal cervical cytology. However, two recent studies have shown that this approach has a much lower sensitivity than previously recognized. In the U.S. multicenter ASCUS/LSIL Triage Study (ALTS), a single colposcopic examination failed to detect 33% to 36% of women who were subsequently identified as having biopsy-confirmed high-grade cervical intraepithelial neoplasia (CIN 2,3) [1,2]. Similarly, in a study from China colposcopy failed to detect 40% of CIN 2,3 lesions [3].

Quantitative optical spectroscopy and imaging offer considerable promise as an approach to improving the performance of colposcopy [4]. The optical methods currently being developed include intrinsic tissue fluorescence, white light backscatter, and video imaging techniques [4]. These methods exploit the biochemical and structural changes that occur within tissue during the pathogenesis of neoplasia to produce a probabilistic prediction of tissue type. Several studies that have
utilized research prototypes have demonstrated that optical methods can be used to classify cervical tissue as being either normal or as having CIN [5–11]. Therefore, optical methods may offer an objective, reproducible result that may improve the sensitivity of colposcopy.

To assess the impact that an optical detection system (ODS) would have on the performance of colposcopy in clinical practice, we conducted a two-arm randomized controlled clinical trial comparing standard colposcopy with colposcopy assisted by ODS. The ODS evaluated in this study was a pre-commercial device that utilizes intrinsic fluorescence, white light backscattered diffuse reflectance spectroscopy, and cervical video imaging.

Materials and methods

The study was a multicenter randomized clinical trial in which women undergoing colposcopic evaluation were randomly assigned to two arms: colposcopy alone or colposcopy in combination with ODS (Fig. 1). The study involved 13 clinical centers and 51 colposcopists including gynecologists, family practice physicians, advanced practice clinicians, and gynecologic oncologists with a specific interest in cervical neoplasia and colposcopy. Their experience in colposcopy ranged from just having completed their obstetrics and gynecology residency training program to having over 30 years of experience with colposcopy. The study was approved by the local institutional review boards at each site.

Females 18 years or older or of age consent referred for evaluation of an abnormal cervical cytology result of atypical squamous cells or greater were eligible for enrollment. Exclusion criteria included pregnancy, current menstruation, previous hysterectomy, cervical stenosis, history of diethylstilbestrol exposure, therapeutic procedure since the referral cervical cytology, cervical cytology tests within the prior seven days, use of vaginal medications within the last 48 h or photosensitizing agents within 72 h, history of photosensitivity or other diseases affected by UV radiation, or an observable and untreated gynecological infection.

Pre-commercial optical detection system devices (MediSpectra Inc., Lexington, MA) were used in this study [12]. Each consisted of a console, display monitor, a “non-contact” illumination probe, and a disposable probe cover. Optical sources include a 337-nm UV nitrogen laser to induce fluorescence and two xenon flash lamps to provide broadband white light for reflectance measurements and video imaging. The optical sources are coupled through a fiber optic cable to the optical probe, where a scanning system selectively illuminates the cervix. Scans of the cervix are performed by interrogating a 25-nm diameter circular area of the ectocervix in a dense grid pattern that includes 499 regions at an approximately 1-mm resolution using laser-induced fluorescence spectroscopy, white light diffuse reflectance spectroscopy, and video imaging. The cervix is scanned by the illumination probe, which is positioned near the proximal opening of the speculum following the application of acetic acid. The scan is a hands-free operation that takes approximately 12 s.

The algorithm employed in this study was determined in a separate pilot clinical study as previously described [12]. The prior pilot study enrolled 604 patients at 6 clinical sites and describes the algorithm development procedure based on pathology-confirmed punch biopsies from 438 subjects together with loop-excisional biopsy samples from an additional 133 subjects. The pilot study subjects were distinct from those enrolled in this study. The algorithm uses a logistic classification based on fluorescence spectra combined with a multivariate statistical analysis method based on the diffuse reflectance spectra. Characteristic wavelengths in the fluorescence spectra (360 nm to 590 nm) were used to determine thresholding rules using absolute and relative spectral intensities to produce a likelihood measure of normal squamous tissue. The diffuse reflectance data (370 nm to 650 nm) were analyzed using singular value decomposition using both principal component and feature extraction analysis techniques. The two techniques are complementary: whereas the principal component method extracts data on the basis of orthogonal vectors that span directions of maximum variance, the feature extraction method extracts data on the basis of vectors that maximize discrimination between data classes. In both cases, measures of data similarity were developed from pathology-verified tissue classes based on the Mahalanobis distance calculation as previously described [12,13]. The two likelihood measures are multiplicatively combined and weighted by the results from the prior fluorescence qualification. The result is an overall likelihood that each tissue area analyzed is characteristic of CIN 2+. This final result is represented as a false color overlay (from light yellow to intense blue) on top of an image of the cervix that represents the increasing likelihood of CIN 2+ throughout the ectocervix (Fig. 2). The ODS system has a built in calibration system to assure that the device is correctly functioning. Investigators received approximately 60 min hands-on training with the device prior to beginning the study. Complete details of the system and the classification algorithm employed are described more fully elsewhere [14].

After insertion of a vaginal speculum, the cervix was washed with a 3% to 5% acetic acid solution, the ODS probe was positioned at the introital opening of the speculum, and an optical scan obtained (Fig. 2). The scan could be initiated 30 to 120 s after the application of the acetic acid solution. The ODS probe is then moved aside and a colposcopic examination initiated. In the combined colposcopy and ODS arm, the ODS result was displayed to the colposcopist who was instructed to take at least one biopsy from any area identified as a high

Fig. 1. Dual-arm study protocol. The study was designed as a dual-arm randomized study in which women in one arm underwent colposcopy without access to the results of the ODS examination, whereas in the other arm colposcopy was performed while being able to view the ODS results (colposcopy plus ODS arm). Biopsies were obtained only in patients in whom either a lesion is identified at the time of colposcopy or in whom the ODS results indicate that a biopsy is required (colposcopy plus ODS arm only).
probability for CIN 2,3 based on the false color overlay, as well as any other clinically indicated biopsies. Therefore, not all women received a cervical biopsy. Each biopsy was processed separately and the colposcopist indicated on the case record form whether the biopsy was obtained based on the ODS result, the colposcopic appearance, or both. In the colposcopy-only arm, the ODS results were not displayed and biopsies were taken solely based on the colposcopist’s clinical judgment.

Cervical biopsies were processed at the clinical sites and reviewed independently by at least two reference pathologists in a blinded fashion. The study endpoint for histopathological diagnosis was CIN 2,3 or cervical cancer (CIN 2+) that was diagnosed using standard histopathologic criteria [15]. If the diagnoses made by the two reference pathologists did not agree, the biopsy was reviewed by a third reference pathologist in order to obtain a majority opinion. The expert pathology panel downgraded to “no CIN 2+” 24% of women (166 of 689) diagnosed as having CIN 2+ by the clinical site pathologists and upgraded to CIN 2+ (2142 (59 of 1880) of women diagnosed as “not CIN 2+” by the clinical site pathologists. At the biopsy level, 2142 (83%) of the 2569 biopsies were given the same diagnosis by the initial two reference pathologists and only 427 (17%) required review by a third reference pathologist. It is now widely appreciated that the histological diagnosis of CIN 1 is quite poorly reproducible and that the majority of CIN 1 lesions represent nothing more than transient HPV infections and therefore are clinically inconsequential [16,17]. Therefore, CIN 1 was not used as an endpoint in this study.

The study was designed to enroll approximately 2200 subjects into the two study arms in a 1:1 ratio. The sample size calculations assumed a loss of 20% of enrolled subjects, in that the prevalence of CIN 2+ in the study population is 35% which is based on data from ALTS and College of American Pathologists, a superiority test design (one-sided test with 2.5% type I error and 90% power) to compare true-positive (TP) rates between the arms, and use of 95% two-sided confidence intervals for the comparison of false-positive (FP) rates between arms [18,19]. For the TP comparison, the null hypothesis was that ODS plus colposcopy would be the same as colposcopy whereas the alternative hypothesis was that ODS plus colposcopy will be at least 6.6% better than colposcopy. For the FP comparison, the study was designed to detect a difference in FP rates of 8% with 90% power. These values were a priori deemed to be clinically meaningful based on conversations with experienced colposcopists.

Randomization was based on a computer generated randomization table using the permuted block method to maximize balance within cytology result strata of first-time ASC, repeat ASC, low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL). Randomization occurred separately at each study center and for each participating investigator.

The per-protocol population is defined as the subset of the intent-to-diagnose population who were randomized, completed the protocol, met all eligibility criteria, and, if undergoing at least one study directed biopsy, had a definitive central pathology finding. Device malfunctions in either study arm that resulted in a lost or invalid scan excluded the patient from the per-protocol population. The primary study endpoints were the subject level true-positive (TP) and false-positive (FP) rates for biopsy-confirmed CIN 2+ in the two groups in the per-protocol population. The TP rate was defined as the number of subjects with at least one positive biopsy (CIN 2+) divided by the total number of subjects from that arm in the per-protocol population. The TP rate is directly proportional to sensitivity. The FP rate was defined as the number of subjects whose biopsies were not CIN 2+ divided by the total number of subjects from that arm in the per-protocol population. Secondary endpoints were the TP and FP rates in the two groups by entry cervical cytology result and associated biopsy-level outcomes.

Joint inference analyses were undertaken to evaluate the significance of the observed changes in TP between the two arms in the context of the observed changes in FP. For these analyses, we considered patient-level outcome is a trichotomy: one or more biopsies taken with one or more biopsies positive (TP), one or more biopsies taken with no biopsies positive (FP), or no biopsy taken (nulls).

The fundamental data structure is a 2×3 frequency table of arm by the trichotomous outcome and the Grizzle–Starner–Koch (GSK) method was used as the primary analysis methodology [20]. GSK produces results consistent with other methods used to analyze frequency tables but also provides extensions for modeling frequency tables with explanatory covariates. The primary statistical test was a stratified joint test for between-arm differences in the proportion of patients with TP and the proportion of patients with FP. The planned overall type I error probability was two-sided 0.05. A significant joint test for the primary analysis would then be followed by characterization of the outcome with respect to assessing whether the outcome is consistent with a TP advantage for ODS and colposcopy arm that is not accompanied by a notably higher FP proportion (<8%). A pre-planned interim analysis to evaluate the study assumptions was conducted based on data from the first 250 per-protocol patients. This interim analysis indicated that the original estimate of the underlying CIN 2+ prevalence of 35% was high, and it was subsequently changed to 33% with the consequence that the formal stopping rule was reduced to the observation of 438 CIN 2+ women in the trial.

Results

The final per-protocol populations were 1096 in the colposcopy-only arm and 1090 in the colposcopy plus ODS...
A total of 37 women were excluded because of device malfunctions (1.6% of all patients). In the majority of these (n=25) the problem was a result of software errors. The software was revised and in the last 1300 women enrolled no similar errors occurred. The other major reason for device malfunction (n=10) was a failure of the device to calibrate after multiple attempts. Baseline characteristics, including referral cytology result, were similar between the two arms (Table 1). ASC, which includes both ASC-US and ASC-H, accounted for 36.3% and LSIL accounted for 43.6% of all study subjects.

Among all patients enrolled in the study, a total of 218 cases of biopsy-confirmed CIN 2+ was identified among the 1096

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**Table 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Per-protocol population</th>
<th>Colposcopy only</th>
<th>Colposcopy plus ODS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>1096</td>
<td>1090</td>
<td>2186</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>25th–75th percentile</td>
<td>22–38</td>
<td>22–37</td>
<td>22–37</td>
<td></td>
</tr>
<tr>
<td><strong>Race/ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>485 (44.3%)</td>
<td>494 (45.3%)</td>
<td>979 (44.8%)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>236 (21.5%)</td>
<td>219 (20.1%)</td>
<td>455 (20.8%)</td>
<td></td>
</tr>
<tr>
<td>Native American</td>
<td>14 (1.3%)</td>
<td>11 (1.0%)</td>
<td>25 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>319 (29.1%)</td>
<td>321 (29.4%)</td>
<td>640 (29.3%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>24 (2.2%)</td>
<td>27 (2.5%)</td>
<td>51 (2.3%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>18 (1.6%)</td>
<td>18 (1.7%)</td>
<td>36 (1.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Referral cytology result</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC a</td>
<td>387 (35.3%)</td>
<td>407 (37.3%)</td>
<td>794 (36.3%)</td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td>484 (44.2%)</td>
<td>469 (43.0%)</td>
<td>953 (43.6%)</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>225 (20.5%)</td>
<td>214 (19.6%)</td>
<td>439 (20.1%)</td>
<td></td>
</tr>
</tbody>
</table>

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a Atypical squamous cells (ASC) include both atypical squamous cells of undetermined significance and atypical squamous cells suggestive of high-grade squamous intraepithelial lesion (HSIL). LSIL, low-grade squamous intraepithelial lesion.

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Please cite this article as: Alvarez RD, et al, Effective cervical neoplasia detection with a novel optical detection system: A randomized trial, Gynecol Oncol 2006, doi:10.1016/j.ygyno.2006.08.056
women in the colposcopy-only arm (TP rate of 19.9%) and 238 among the 1090 women in the colposcopy plus ODS arm (TP rate of 21.8%), as seen in Table 2. This corresponds to a 9.8% gain in TP rate (p=0.143). In the colposcopy-only arm, 629 of the 1096 women had a cervical biopsy in which CIN 2+ was not identified (FP rate of 57.4%) compared to 659 women of the 1090 women in the colposcopy plus ODS arm (FP rate of 60.5%). Cervical biopsies were taken in 847 (77.3%) of women enrolled in the colposcopy-only arm compared to 897 (82.3%) of the women in the colposcopy plus ODS arm. Thus, overall, 5% more women received biopsies in the colposcopy plus ODS arm and 2% more cases of CIN 2+ were identified compared to the colposcopy-only arm.

When results were stratified by referral cervical cytology, a 26.8% gain in TP rate was observed in the colposcopy plus ODS arm compared to colposcopy alone among women referred for the evaluation of ASC (Table 3). A similar increase in TP rate was observed among the women referred for LSIL. Women with an ASC or LSIL referral cytology accounted for 79.9% of the entire study population and when these two groups are considered together, the overall TP rate increased from 11.4% in the colposcopy-only arm to 14.4% in the colposcopy plus ODS arm (Table 3). This represents a 26.5% gain in TP rate obtained using colposcopy plus ODS (p=0.035). TP rates were similar between the two arms among women referred for the evaluation of HSIL.

The clinical setting for the evaluation of an adjunct to colposcopy is inherently limited because the unknown prevalence of disease in the patients studied means the sensitivity of the adjunct cannot be directly evaluated. However, by assuming a range of prevalence values for disease in the population, the TP results from this study can be transformed to show the impact on sensitivity. Fig. 4 shows for women with ASC or LSIL the estimated difference in sensitivity and corresponding projected confidence intervals for assumed values of prevalence of CIN 2+. The figure demonstrates a consistent 10–20% absolute sensitivity advantage for colposcopy plus ODS compared to colposcopy alone over a wide range of possible prevalence values for women with ASC or LSIL.

FP rates (biopsy taken but no CIN 2+ identified) were high in both arms of the study (Table 2). Among all women enrolled in the colposcopy-only arm, the FP rate was 57.4% and in the

Table 2
True-positive, false-positive patients, and patients without biopsy by referral pap and study arm

<table>
<thead>
<tr>
<th>Referral cytology</th>
<th>Colposcopy-only arm (n, %)</th>
<th>Colposcopy plus ODS arm (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
<td>FP</td>
</tr>
<tr>
<td>ASC</td>
<td>42</td>
<td>210</td>
</tr>
<tr>
<td>LSIL</td>
<td>57</td>
<td>323</td>
</tr>
<tr>
<td>HSIL</td>
<td>119</td>
<td>96</td>
</tr>
<tr>
<td>Total</td>
<td>218</td>
<td>629</td>
</tr>
<tr>
<td>ASC/LSIL</td>
<td>99</td>
<td>533</td>
</tr>
</tbody>
</table>

* A true-positive (TP) is defined as all women having biopsy-confirmed CIN 2+ or cancer. A false-positive (FP) is defined as all women having at least one cervical biopsy taken with none of the biopsies diagnosed as CIN 2+ or cancer. Percentages are row percentages by arm for each referral cytology strata.

b Atypical squamous cells (ASC) include both atypical squamous cells of undetermined significance and atypical squamous cells suggestive of high-grade squamous intraepithelial lesion (HSIL). LSIL, low-grade squamous intraepithelial lesion.

Table 3
Differences and percent gain of biopsy-confirmed CIN 2+ or cancer by referral pap and study arm

<table>
<thead>
<tr>
<th>Referral pap</th>
<th>Percentage with CIN 2+</th>
<th>Difference</th>
<th>%TP Gain</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colposcopy only</td>
<td>Colposcopy plus ODS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>10.9%</td>
<td>13.8%</td>
<td>2.9%</td>
<td>+26.8%</td>
</tr>
<tr>
<td>LSIL</td>
<td>11.8%</td>
<td>14.9%</td>
<td>3.1%</td>
<td>+26.7%</td>
</tr>
<tr>
<td>HSIL</td>
<td>52.9%</td>
<td>52.3%</td>
<td>-0.6%</td>
<td>-1.0%</td>
</tr>
<tr>
<td>Total</td>
<td>19.9%</td>
<td>21.8%</td>
<td>1.9%</td>
<td>+9.8%</td>
</tr>
<tr>
<td>ASC/LSIL</td>
<td>11.4%</td>
<td>14.4%</td>
<td>3.0%</td>
<td>+26.5%</td>
</tr>
</tbody>
</table>

p-values are one-sided Fisher’s exact test.

Fig. 4. Estimated difference in sensitivity for assumed values of CIN 2+ prevalence. The estimated difference in sensitivity of colposcopy plus ODS compared to colposcopy alone was calculated for a given prevalence of CIN 2+ among women referred for evaluation of an ASC or LSIL cytology result. Based on the literature the prevalence of CIN 2+ among women with ASC ranges from 5% to 17% and the prevalence among women with LSIL ranges from 15% to 30% [37]. Therefore, the prevalence of CIN 2+ among women with ASC or LSIL combined was varied from 15% to 29%. Exact confidence intervals were computed using the observed number of TP cases in each arm as the numerator and the projected number of cases in each arm as computed from the assumed prevalence as the denominator.
colposcopy plus ODS arm it was 60.5%. In the clinically important ASC/LSIL strata, the FP rates were 61.2% in the colposcopy-only arm and 65.2% colposcopy plus ODS arm, remaining below the 8% difference statistical assumption. The average number of biopsies for all patients in the two arms was 1.03 for the colposcopy-only arm and 1.30 in the colposcopy plus ODS arm (Table 4). This represents an average increase of 0.27 biopsies per patient in the colposcopy plus ODS arm. The number of biopsies taken per patient varies considerably depending on the referral cytology. The average number of biopsies in the colposcopy-only arm was 0.81 among women whose referral cytology was ASC and 1.40 among those referred for evaluation of HSIL. These increased to 1.15 and 1.57, respectively, in the colposcopy plus ODS arm (Table 4).

In the colposcopy plus ODS arm, the colposcopist declared whether a biopsy was obtained based solely on the colposcopic appearance, based solely on the ODS result, or whether a biopsy was indicated by both colposcopy and ODS. Based on this declaration the positive predictive value (PPV) of biopsies indicated by the ODS result could be compared with that for biopsies indicated by colposcopy. For women with ASC or LSIL, the PPV of biopsies indicated by ODS was 15.0% (110/734) and the PPV of biopsies indicated by colposcopy was 15.2% (125/825). Biopsies indicated by both the ODS result and colposcopy had the highest PPV at 18.1% (89/491). For comparison, the PPV of the colposcopically directed biopsies in the colposcopy-only arm among women with ASC or LSIL was 14.1%.

A significant arm by stratum interaction was found to exist in the stratified GSK model ($p=0.0175$). Data inspection following the discovery of this interaction led to the conclusion that the HSIL stratum was the source of the across strata heterogeneity and therefore further analyses should be focused on the data remaining after deleting the data from the HSIL stratum. Dropping the HSIL stratum is data driven and therefore requires that the significance level be lowered to 0.00714 for subsequent analyses focused on the primary objective.

The stratified between-arm test using the data remaining after deleting the HSIL stratum has a $p$-value of 0.0018, meeting the adjusted significance criterion. The TP difference estimate from this model is 2.78% and the FP difference estimate is 4.00%. The TP difference estimate indicates an ODS and colposcopy arm advantage, and the estimated FP difference is not close to the pre-specified adverse criterion of 8%.

Therefore, it can be concluded that colposcopy plus ODS provides a benefit compared to colposcopy alone among women with ASC or LSIL.

No significant adverse events were encountered in either arm of the study. Only 4 subjects experienced minor adverse events during the entire study. These include cramps, vomiting/ weakness, minor bleeding, and faintness. None of the minor adverse events were related to the use of ODS.

**Discussion**

This is the first multicenter randomized trial to evaluate the clinical performance of optical detection technology in conjunction with colposcopy to enhance the detection of high-grade cervical neoplasia in women being evaluated for an abnormal cervical cytology. The trial was designed to evaluate the use of a pre-production commercial ODS system as it would be used in a real practice clinical setting. Use of ODS in conjunction with colposcopy resulted in a 26.5% increase in the detection of biopsy-confirmed CIN 2+ among women referred for the evaluation of an ASC or LSIL cervical cytology result. Although the increase in TP is of marginal statistical significance ($p=0.035$, one sided), the 26.5% increase in detection of CIN 2+ has clinical significance. Moreover, the increase occurred with only a modest 4% increase in FP rate and joint hypothesis testing that takes into account both the TP rate and the FP rate indicates that colposcopy plus ODS provides a benefit compared to colposcopy alone among women with ASC or LSIL.

Colposcopy is a highly subjective clinical science, with the accuracy dependent upon the training and experience of the colposcopist [21–23]. For many years, it was thought that colposcopy is a sensitive, but rather non-specific, method for identifying high-grade cervical neoplasia. A 1998 comprehensive meta-analysis of the performance of colposcopy estimated that it had a weighted mean sensitivity for distinguishing normal tissue from abnormal tissue of 0.96 (95% CI 0.95–0.97) and a weighted mean specificity of 0.48 (95% CI 0.47–0.49) [24]. However, several studies have reported false-negative rates for colposcopy ranging from 15% to 31% for CIN 2,3 lesions [25]. The recent ALTS trial found that even when highly trained clinicians performed colposcopy under carefully monitored circumstances only 64% of the cumulative cases of biopsy-confirmed CIN 2,3 were identified at an initial colposcopic

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of biopsies per patient by arm and by referral cytology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Referral pap*</th>
<th>Colposcopy only</th>
<th></th>
<th></th>
<th>Colposcopy plus ODS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of biopsies</td>
<td>Number of patients</td>
<td>Patients without biopsy</td>
<td>Average biopsies per patient</td>
<td>Number of biopsies</td>
<td>Number of patients</td>
<td>Patients without biopsy</td>
</tr>
<tr>
<td>ASC</td>
<td>313</td>
<td>387</td>
<td>135</td>
<td>0.81</td>
<td>470</td>
<td>407</td>
<td>97</td>
</tr>
<tr>
<td>LSIL</td>
<td>500</td>
<td>484</td>
<td>104</td>
<td>1.03</td>
<td>614</td>
<td>469</td>
<td>82</td>
</tr>
<tr>
<td>HSIL</td>
<td>315</td>
<td>225</td>
<td>10</td>
<td>1.40</td>
<td>336</td>
<td>214</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>1128</td>
<td>1096</td>
<td>249</td>
<td>1.03</td>
<td>1420</td>
<td>1090</td>
<td>193</td>
</tr>
<tr>
<td>ASC/LSIL</td>
<td>813</td>
<td>871</td>
<td>239</td>
<td>0.93</td>
<td>1084</td>
<td>876</td>
<td>179</td>
</tr>
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* Atypical squamous cells (ASC) include both atypical squamous cells of undetermined significance and atypical squamous cells suggestive of high-grade squamous intraepithelial lesion (HSIL). LSIL, low-grade squamous intraepithelial lesion; CIN 2+, CIN 2,3, or invasive cervical cancer.
examination in women with preceding ASC results.[1] Similarly, only 67% of the cumulative cases of biopsy-confirmed CIN 2,3 among women with LSIL were identified at initial colposcopy [2]. A cervical screening study of 1997 women conducted in Shanxi China by Belinson et al. [26] confirms the low sensitivity of colposcopy observed in ALTS. In the Shanxi study women received colposcopy and had cervical biopsies taken of all areas classified as abnormal by colposcopy. In addition, random four quadrant cervical biopsies were obtained from colposcopically normal appearing regions of the cervix. In that study, the sensitivity of colposcopy for identifying CIN 2,3 was 78%. A second study from Shanxi China that also utilized random cervical biopsies reported that the sensitivity of colposcopy for identifying CIN 2+ among women with an ASC or LSIL cytology result is 60% [3]. In the current study, 57% of women without CIN 2+ had at least one cervical biopsy obtained in the colposcopy-only arm as did 61% in the colposcopy plus ODS arm. This appears to be within the range of what is typically observed in routine clinical practice. For example, in a study from Kaiser Southern California of women undergoing colposcopy for ASC cytology 76% of women without CIN 2+ were biopsied during the course of routine colposcopy [27].

Different forms of spectroscopic analysis are being developed as diagnostic modalities for a variety of organ sites. Fluorescence spectroscopy is the most widely studied and promising results have been shown for identifying neoplasia of the colon, cervix, bronchus, bladder, and other sites [28]. The cervix may be a particularly suitable site for utilizing spectroscopic analysis since it is easily accessible and cervical neoplasia is relatively common. Much of the early work on using fluorescence spectroscopy to diagnose cervical neoplasia was carried out by Richards-Kortum and co-workers. In the mid-1990s, this group developed multivariate algorithms that utilized fluorescence emission spectra and tested these algorithms in diagnostic and screening populations [6,29–31]. More recently, other groups have attempted to overcome the limitations inherent in fluorescence spectroscopy by combining it with other forms of spectroscopic analysis. Georgakoudi et al. combined fluorescence spectroscopy together with diffuse reflectance and light scattering and found that the combination of techniques was superior to any single technique for the detection of CIN [10]. In a study of 44 subjects referred for the evaluation of an abnormal cervical cytology, they reported a sensitivity of 92% and a specificity of 71% for differentiating biopsy-confirmed CIN from non-CIN utilizing the three techniques in combination. In another study that used a combination of fluorescence spectroscopy and reflectance measurements, Ferris et al. reported an optimized sensitivity of 95% and an optimized specificity of 86% by receiver operator curve (ROC) analysis for CIN 2+ [32]. Other investigators have constructed neural nets for the analysis of fluorescence spectral data and in relatively small studies have been able to achieve average correct classification rates of 96.5% for CIN 1 [9].

The current trial expands these previous studies in a number of important ways. It is the first randomized clinical trial to assess combining spectroscopic analysis with colposcopy. Moreover, the trial was quite large and the ODS units that were used were commercial, pre-production units utilizing a fully locked algorithm rather than single prototype research units. The ODS units utilized in this study image the entire visible portion of the cervix in most women without contacting the cervix and require only 12 s for image acquisition. The design of the trial allowed us to evaluate enhancements in the performance of colposcopy with the use of ODS at a subject level rather than simply at an individual biopsy level to which the smaller previous research studies have been restricted.

The results of the current study clearly demonstrate that the use of ODS in conjunction with colposcopy will allow a considerable proportion of cases of CIN 2,3 that are missed by colposcopy alone to be detected in women with ASC and LSIL referral cytology. The gain in detection of biopsy-confirmed CIN 2+ in the ASC/LSIL population came at the expense of an average of 0.3 additional biopsies per patient. Moreover, the increased detection of CIN 2+ in the colposcopy plus ODS arm does not appear to be due to a “random biopsy” effect. In the ASC/LSIL population, the positive predictive value of biopsies obtained based on the ODS result was nearly identical to that of biopsies obtained based on colposcopic appearance in the colposcopy plus ODS arm.

The finding that the ODS did not increase the detection of CIN 2,3 among women referred for the evaluation of HSIL cervical cytology may be due to several factors. One is that CIN 2,3 lesions are significantly larger among women referred for the evaluation of an HSIL cytology result compared to ASC or LSIL [33–35]. This would be expected to make the lesions easier to identify since lesion size correlates with the accuracy of the colposcopic impression [36]. Another factor is whether or not the colposcopist expects to identify a CIN 2,3 lesion. In the ALTS clinical trial, more cases of CIN 2,3 were identified by colposcopy when the clinician knew the women were high-risk HPV DNA positive [1]. The lack of improvement in detection of CIN 2,3 with ODS among women referred for evaluation of HSIL is of little importance clinically as recent management guidelines recommend that these women undergo a diagnostic excisional procedure whenever CIN 2,3 is not identified [19,37].

Increasing the performance of colposcopy through the routine use of an ODS system could have important clinical benefits. With current practice guidelines, approximately 2 to 2.5 million women with ASC or LSIL require a colposcopic evaluation each year in the United States [38]. The ALTS clinical trial found that approximately 27% of these women, or about 600,000 women, have CIN 2,3 and that a single colposcopic examination would identify only approximately two-thirds of these cases [38]. Based on the results of the current study, the widespread adoption of ODS as an adjunct to colposcopy would identify 26.5% more cases of CIN 2,3 than colposcopy alone. This would result in the identification of over 100,000 cases of CIN 2,3 that would have not been detected at their initial colposcopic examination.

**Acknowledgment**

The study was funded by MediSpectra, Inc., Lexington, MA.
Appendix A

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References
[18] Human papillomavirus testing for triage of women with cytologic evidence...


[38] Cox JT, Schiffman M, Solomon D. 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;188:1406–12.