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Research Article

Cancer Prevention Research

Diagnosing Cervical Neoplasia in Rural Brazil Using a Mobile Van Equipped with *In Vivo* Microscopy: A Cluster-Randomized Community Trial

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Abstract

Cervical cancer is a leading cause of death in underserved areas of Brazil. This prospective randomized trial involved 200 women in southern/central Brazil with abnormal Papanicolaou tests. Participants were randomized by geographic cluster and referred for diagnostic evaluation either at a mobile van upon its scheduled visit to their local community, or at a central hospital. Participants in both arms underwent colposcopy, *in vivo* microscopy, and cervical biopsies. We compared rates of diagnostic follow-up completion between study arms, and also evaluated the diagnostic performance of *in vivo* microscopy compared with colposcopy. There was a 23% absolute and 37% relative increase in diagnostic follow-up completion rates for patients referred to the mobile van (102/117, 87%) compared with the central hospital (53/83, 64%; $P = 0.0001$; risk ratio = 1.37, 95% CI, 1.14–1.63). In 229 cervical sites in 144 patients, colposcopic examination identified sites diagnosed as cervical intraepithelial neoplasia grade 2 or more severe (CIN2+; 85 sites) with a sensitivity of 94% (95% CI, 87%–98%) and specificity of 50% (95% CI, 42%–58%). *In vivo* microscopy with real-time automated image analysis identified CIN2+ with a sensitivity of 92% (95% CI, 84%–97%) and specificity of 48% (95% CI, 40%–56%). Women referred to the mobile van were more likely to complete their diagnostic follow-up compared with those referred to a central hospital, without compromise in clinical care. *In vivo* microscopy in a mobile van provides automated diagnostic imaging with sensitivity and specificity similar to colposcopy. *Cancer Prev Res; 11(6); 359–70. ©2018 AACR.*

Introduction

Cervical cancer remains a major global public health problem despite effective screening with Papanicolaou (Pap) and human papillomavirus (HPV) DNA testing as well as the more recent introduction of the HPV vaccine. Over 500,000 new cases and 270,000 deaths due to cervical cancer still occur annually worldwide (1). Over 85% of cases and deaths occur in women in low- and middle-income countries (LMIC), where Pap- or HPV DNA-based screening and follow-up programs are often lacking or flawed (1–3).

In Brazil, approximately 16,400 new cases of cervical cancer occur each year, representing the third highest cancer incidence among Brazilian women, excluding non-melanoma skin cancer (4). Incidence rates vary within Brazil with the highest rates in underserved areas located far from screening and diagnostic centers (4). One strategy to reach patients in these areas, developed by Barretos Cancer Hospital in the northern part of São Paulo state, is the use of medically equipped semi-trailer trucks that travel to underserved communities to perform Pap-based cervical cancer screening and other services (5–11). While this is effective in terms of initial screening, patients with an abnormal Pap test result must still travel to a central

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hospital for diagnostic follow-up (colposcopy, biopsy, and histopathologic evaluation)—often a journey of 2 or more days by bus. It is estimated that up to two-thirds of screen-positive women fail to return to the central hospital for the necessary diagnostic workup and treatment, especially when they live far from the central facility (8).

In this study, we explored a strategy for bringing mobile diagnostic follow-up to underserved communities in Brazil. This was achieved through the use of a mobile diagnostic van equipped with a colposcope, *in vivo* microscope, cryotherapy equipment, and staffed by a colposcopist and a research nurse. The mobile diagnostic van traveled from Barretos Cancer Hospital to communities where patients with abnormal Pap results had previously been identified via mobile screening trucks. Patients received diagnostic follow-up care in the van including colposcopy, *in vivo* microscopy, and cervical biopsies. The *in vivo* microscope is an important component of this strategy and consists of a low-cost fiber-optic imaging device called the high-resolution microendoscope (HRME) that can image subcellular morphologic features in real time (12, 13). This gives the clinician the ability to evaluate the size, shape, and distribution of cell nuclei *in situ* at the point of care—information that is typically only available weeks to months later after taking a biopsy, tissue processing, and evaluation at the central hospital. This real-time diagnostic imaging capability could potentially be used to help guide the selection of biopsy sites and reduce the number of biopsies needed for diagnosis. It could also potentially help identify lesions that are suitable for immediate treatment using cryotherapy, minimizing losses to follow-up.

The primary objective of this study was to compare rates of diagnostic follow-up completion for screen-positive women randomized to attend a mobile diagnostic van in their local community versus a central hospital for diagnostic follow-up. The secondary objective of the study was to evaluate the diagnostic performance of *in vivo* microscopy compared with colposcopy using histopathology as the gold standard.

Materials and Methods

Study design for evaluation of mobile diagnostic follow-up

We conducted a non-blinded, cluster-randomized community trial to evaluate two strategies for follow-up diagnosis of cervical cancer precursor lesions in patients with an abnormal Pap test result: (i) referral of patients to a mobile diagnostic van upon its visit to their local community (experimental arm) versus (ii) referral of patients to travel to a central hospital for diagnostic follow-up (standard-of-care arm). Subjects were randomized by geographic cluster to the experimental arm or the standard-of-care arm.

The study was approved by the Barretos Cancer Hospital Ethics Research Committee, the Brazilian National Ethics Research Commission (CAAE: 37774314.3.0000.5437), and the institutional review boards of Rice University (ID#653693) and the University of Texas MD Anderson Cancer Center (ID#2015-0442). Written informed consent was obtained from all participants. The protocol was registered with ClinicalTrials.gov (NCT 02494310).

Participants

Potential subjects were identified through a regional Pap Smear screening program operated by Barretos Cancer Hospital (BCH) via mobile screening trucks. Each city scheduled to be visited by the mobile screening trucks was eligible for cluster randomization. Randomization was done in three rounds as the schedule for the mobile screening trucks was not known in advance. In each round, a list of cities was provided by clinicians at BCH to collaborators at Rice University. Each city was assigned a number and a computer program was used to generate a random number sequence for the number of cities. The first half of the cities in the sequence was assigned to the experimental arm, whereas the second half was assigned to the standard care arm. A total of 234 geographic clusters were randomized; 79 clusters with patients having abnormal Pap results were included in the study (Supplementary Table S1). Per Brazilian guidelines, women 18 years of age or older who had an abnormal screening Pap test (≥ASC-US) were referred for colposcopy and therefore eligible for the study and invited by telephone to participate.

Each geographic cluster was characterized by its distance from the central hospital (BCH) and by its human development index (HDI), which is a composite statistic of life expectancy, education, and per capita income. HDI values for each geographic cluster/municipality were obtained from an official Brazilian government website, Instituto Brasileiro de Geografia e Estatistica, at: https://cidades.ibge.gov.br/xtras/home.php, accessed May 2016.

Diagnostic setting

Patients in the standard-of-care arm traveled to the colposcopy clinic at BCH for the required diagnostic procedures. Patients in the experimental arm underwent diagnostic procedures in a mobile diagnostic van that traveled to their community (Fig. 1). The interior of a Mercedes Sprinter 515 CD1 van was configured to include two rooms: a changing room in the rear of the vehicle and an examination room equipped with a table for gynecologic examinations, colposcope, *in vivo* microscope, supplies, washbasin, electrical power (when plugged in to an external power source), running water, and air conditioning. All vehicle adaptions were made in the Mobile Unit Factory of BCH (Barretos Lamboo Company).

In vivo microscopy

The HRME was developed at Rice University (Houston, Texas, USA). It is a portable, battery-powered fluorescence microscope with a flexible fiber-optic probe (14–16). The HRME has a field of view of 790 microns and transverse

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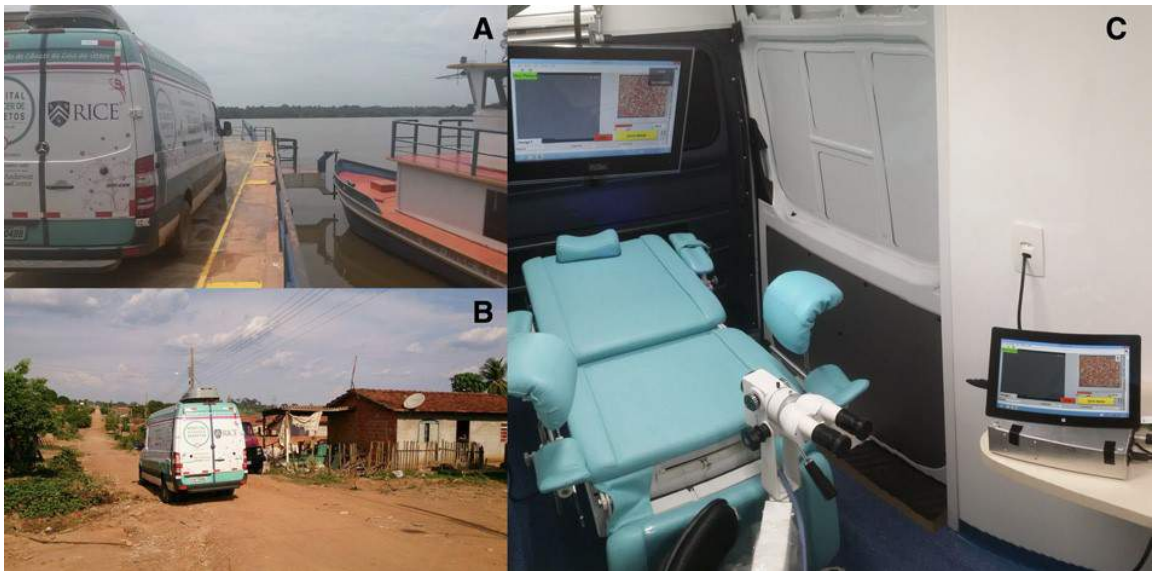

Figure 1.
The mobile diagnostic van. **A,** *In transit* at the Juruena river; **B,** arriving at a remote community in the state of Mato Grosso; **C,** view of the examination room inside the van.

resolution of 4 microns; the probe is flexible but has a minimum recommended radius of curvature of 90 mm. The HRME is used in combination with the topically applied contrast agent 0.01% proflavine. During use, the clinician holds the HRME probe a few inches back from the distal tip and places the probe in contact with the tissue surface. The HRME device images the size, shape, and distribution of epithelial cell nuclei.

Upon image acquisition, the HRME software automatically processes the image, performs a quality control check, and applies a quantitative diagnostic algorithm based on morphologic features including the size, eccentricity, and crowding of cell nuclei (17–20). The automated algorithm segments individual nuclei and outlines them in the image display in yellow if they fall within a normal range for size and eccentricity as defined in the software, or in red if they exceed those limits. After segmenting the image in this manner, the quality control check is performed using two quantitative metrics: raw pixel intensity of the fiber bundle region and signal to background ratio of the segmented nuclei compared with the segmented cytoplasm areas of the image. Finally, the automated algorithm calculates a quantitative parameter defined as the number of abnormal nuclei per square millimeter of area included in the analyzed field of view. This parameter, #abnormal nuclei/mm^2, is used to categorize the overall image as nonneoplastic or neoplastic, with a preestablished threshold value of 120 abnormal nuclei/mm^2 as the cutoff. The threshold values for quality control and diagnosis were established based on data from a previous cervical dysplasia imaging study (13) and data from the first 34 patients in this study (22 in the central facility arm and 12 in the mobile van arm). Starting with the 35th patient in this study, the algorithm, parameters, and thresholds were fixed throughout the remainder of the study. The HRME displays this quantitative result and a diagnostic prediction for the site within approximately 6 to 8 seconds following image acquisition.

Diagnostic procedures

Diagnostic procedures were carried out in an identical fashion in the central hospital and the mobile van. Procedures were performed by one of three colposcopists with similar training and clinical experience (JCPR, MA, BOF). All three colposcopists participated in both arms of the study.

An initial examination was performed using visual inspection with 5% acetic acid followed by colposcopic examination. Colposcopy images were acquired using a KLP 210 colposcope (Kolplast ci Ltda) in the mobile van and CP-M1255 colposcope (DFV) in the central facility. Any colposcopically abnormal areas were identified and recorded as "low grade" (LG), "high grade" (HG), or "cancer". By default, any areas not identified as abnormal were categorized as "no lesion" by colposcopy. Colposcopic calls of low grade or more abnormal were considered positive, consistent with standard of care at BCH. All colposcopy images and impressions were recorded prior to *in vivo* microscopy. Colposcopy images in addition to colposcopic impressions were entered into the study database (REDCap), but only the impressions were used in the diagnostic performance analysis presented.

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After colposcopy, proflavine at a concentration of 0.01% was then applied to the cervix and *in vivo* microscopy was performed using the HRME device. HRME images were acquired from colposcopically abnormal cervical sites (with HRME probe placement at these sites guided by colposcopy). HRME images were also acquired from a minimum of one site per quadrant, even if no colposcopically abnormal lesion was present (with HRME probe placement at these sites guided by visual examination). The colposcopist was tasked with collecting a single HRME image from each site, but had discretion to collect more than one image if desired. If more than one image was collected at a single site, the image with the highest (most abnormal) quantitative score according to the automated algorithm was used. The diagnostic prediction generated by the automated HRME software was noted for each site. Sites identified as abnormal by colposcopy or by HRME imaging were biopsied. If no sites were identified as abnormal by either method, a single biopsy was taken from a clinically normal site randomly selected by the clinician. Endocervical curettage (ECC) was performed in a small number of patients when clinically indicated (mobile van: 9 patients; central facility: 3 patients). Because HRME is not capable of imaging the endocervix, pathology resulting from ECC does not correspond to cervical sites where images were acquired. Therefore, ECC pathology was not used to evaluate diagnostic performance.

Histopathology

Biopsy specimens underwent standard processing with hematoxylin and eosin staining as per standard procedure. Two expert pathologists (G. de Macêdo Matsushita, C. Scapulatempo-Neto, or L. Kerr) who were blinded to all study results reviewed the histologic slides and classified each site as benign (including normal, atrophy, and inflammation), cervical intraepithelial neoplasia grade 1 (CIN1), grade 2 (CIN2), grade 3 (CIN3), adenocarcinoma *in situ* (AIS), or invasive carcinoma, according to standard criteria defined by the World Health Organization (21). Discrepant results were resolved by consensus review.

Treatment

Patients in the standard-of-care arm with a histopathology result of CIN2 or more severe (CIN2+) were referred within the central hospital facility for loop electrosurgical excision procedure (LEEP), cold-knife conization (CKC), or cancer management based on the results. Patients in the standard-of-care arm with histopathology results of <CIN2 were recommended to have follow-up in 6 and/or 12 months per local standard of care. Cryotherapy was not offered to subjects in the standard-of-care arm.

For patients in the experimental arm with a lesion noted by colposcopy, the option of immediate treatment using cryotherapy in the mobile diagnostic van was available, provided the lesion covered less than 75% of the ectocervix, could be fully covered with the cryotherapy tip, did not extend into the endocervical canal, and was not suggestive of cancer (22). These are standard local eligibility criteria for cryotherapy. The assessment of eligibility for cryotherapy was made on the basis of visual inspection and colposcopic imaging. Women with a lesion noted by colposcopy who were ineligible for cryotherapy or who chose not to get cryotherapy were referred to the central hospital facility for follow-up and/or treatment as per standard of care. Women without a lesion noted by colposcopy were recommended to have follow-up. In any case where subsequent histopathology indicated CIN2+, the patient was referred to the central hospital facility for treatment as per standard of care.

Patients in the experimental arm who received cryotherapy but whose histopathology results subsequently indicated AIS or invasive carcinoma were recalled to a central hospital for immediate treatment. Patients who received cryotherapy and whose histopathology results subsequently indicated CIN2 or CIN3 were scheduled for follow-up at the central hospital in 6 months per local standard of care.

Statistical analyses

Sample size calculations for the study design were performed based on a cluster randomization power analysis using the two-sided score test (23). Sampling 20 clusters with five subjects each in both arms provided 81% power ($P = 0.05$) to detect a 30% absolute increase in diagnostic follow-up completion rates for screen-positive women in the experimental arm, assuming that only 40% of the women with an abnormal Pap test would return in the standard-of-care arm. All data were collected and managed using the REDCap (Research Electronic Data Capture) platform (24). Statistical significance testing was performed using *R* 3.2.2 (25).

The primary endpoint was the rate of diagnostic follow-up completion for women in each arm of the study. Diagnostic completion rates were compared using Fisher's exact test. The relative difference in completion rates was quantified using a risk ratio. The risk ratio and the 95% confidence interval (95% CI) of completing diagnostic follow-up were calculated using unconditional maximum likelihood estimation (Wald; ref. 26).

The secondary endpoint was diagnostic performance (sensitivity and specificity) of colposcopy and *in vivo* microscopy for detection of CIN2+. Both low-grade or more abnormal (LG+) and high-grade or more abnormal (HG+) were used as thresholds for positivity to evaluate the diagnostic performance of colposcopy. Sensitivity and specificity with 95% binomial exact confidence intervals were calculated on both a per-site basis and a per-patient basis, with the most abnormal colposcopy result, HRME result, and histopathology result used in the per-patient analysis. Significance testing for the two diagnostic methods for sensitivity and specificity was performed using McNemar's test, which is a statistical

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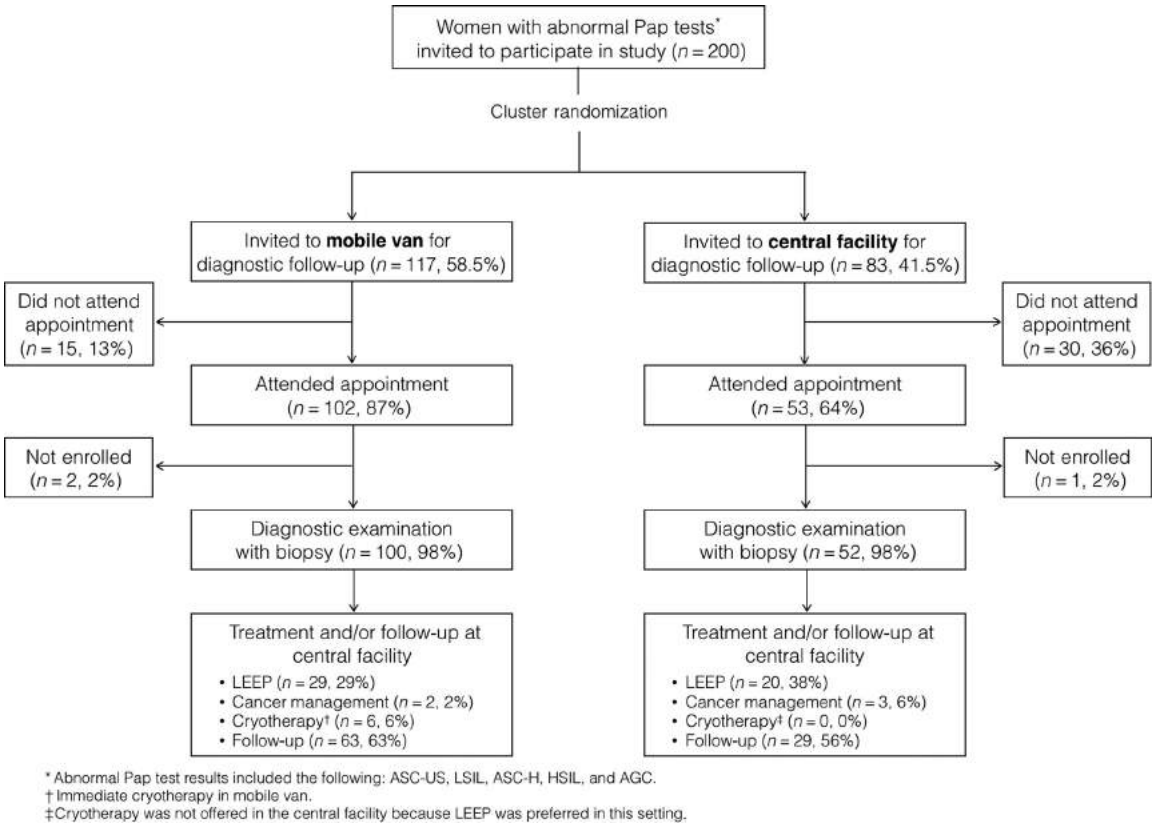

Figure 2.
Comparison of the diagnostic follow-up rates between the mobile van and central hospital facility. Treatment and/or follow-up information reflects management recommendations, which in some cases may differ from treatment and/or follow-up actually received (except in the case of cryotherapy in the mobile van arm, which was performed immediately).

test used to determine whether there are differences in a dichotomous-dependent variable between two related groups (27).

Results

The study schema is shown in Fig. 2. Following cluster randomization, 83 patients from 41 geographic clusters were assigned to the standard-of-care arm (central hospital) and 117 patients from 38 geographic clusters were assigned to the experimental arm (mobile diagnostic van). There were no significant differences between the geographic clusters in the standard of care and experimental arms with regards to mean human development index (0.699 and 0.700, respectively) and mean distance to the central hospital facility (1,020 km and 996 km, respectively) (Supplementary Table S1).

The primary outcome results are shown in Table 1: there was a 23% absolute and 37% relative increase in diagnostic completion rates in the mobile van (102 of 117 patients, 87%) compared with the central hospital facility (53 of 83 patients, 64%; $P = 0.0001$; risk ratio = 1.37, 95% CI, 1.14–1.63). As the risk ratio of 1.37 indicates, the attendance rate for women in the mobile van arm was 37% higher relative to the attendance rate for women in the central hospital arm. Of the 155 patients who presented for diagnostic follow-up, 152 underwent both colposcopy and *in vivo* microscopy (100 patients in the mobile van and 52 patients in the central hospital facility). Three patients who presented for diagnostic follow-up (two in the mobile van

Table 1. Diagnostic completion rates for patients who received diagnostic follow-up at the central hospital facility compared with the diagnostic mobile van

	Number of patients invited	Number (%) of patients attended	P^a	Risk ratio	95% CI
Mobile diagnostic van	117	102 (87%)	0.0001	1.37	1.14–1.63
Central facility	83	53 (64%)			

[a]*P* value calculated using the Fisher's exact test.

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Table 2. Screening Pap results, final biopsy diagnosis, and referral to treatment for the 152 patients who completed diagnostic follow-up

	Mobile van	Central facility	
No. patients	100	52	
Presenting Pap diagnosis, no. patients (%)			***P***[a]
ASC-US	36 (36)	13 (25)	0.18
ASC-H	23 (23)	13 (25)	
LSIL	26 (26)	10 (19)	
HSIL	12 (12)	13 (25)	
AGC	3 (3)	3 (6)	
Final biopsy diagnosis, no. patients (%)			***P***
Benign	40 (40)	17 (33)	0.27
CIN1	30 (30)	13 (25)	
CIN2	14 (14)	7 (13)	
CIN3	11 (11)	14 (27)	
Adenocarcinoma *in situ*	1 (1)	0 (0)	
Invasive carcinoma	2 (2)	1 (2)	
Indeterminate	2 (2)	0 (0)	
Referral to treatment, no. patients (%)			***P***
Follow-up	63 (63)	29 (56)	0.11
Cryotherapy (only offered in mobile van arm)	6 (6)	0 (0)	
LEEP	29 (29)	20 (38)	
Cancer management	2 (2)	3 (6)	

NOTE: Treatment and/or follow-up information reflects management recommendations, which in some cases may differ from treatment and/or follow-up actually received (except in the case of cryotherapy in the mobile van arm, which was performed immediately).

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; LSIL, low-grade squamous intraepithelial lesion.

[a]*P* values calculated using Fisher's exact test. *P* values compare the populations in the two arms of the study in terms of presenting Pap diagnosis, final biopsy diagnosis, and referral to treatment. No statistically significant difference was observed between the populations in terms of those categories ($P > 0.05$).

and one at the central facility) did not undergo these procedures: one underwent LEEP treatment at a different facility, one had excessive bleeding preventing colposcopy, and one had a scheduling error. The presenting Pap findings, final biopsy diagnoses, and referral to treatment for these 152 patients are summarized in Table 2.

A total of 625 cervical sites in these 152 patients were examined using colposcopy and HRME imaging and the results compared with the gold standard of histopathologic diagnosis. We excluded 396 of the cervical sites from the diagnostic performance analyses due to (i) normal findings with no biopsy taken ($n = 350$, 56%), (ii) HRME images failed an automated quality control check ($n = 39$, 6%), and (iii) indeterminate histopathology ($n = 7$, 1%). The majority of images that failed the automated quality control check were those with very low overall intensity, which can arise from poor contact between the fiber probe and cervical tissue. Of the seven cases with indeterminate histopathology, in three cases both pathologists' individual reads indicated an indeterminate result or insufficient tissue for analysis. In four cases, the pathologists' individual reads did not agree and they were unable to reach a clear consensus result upon further review. As a result of the exclusions applied to cervical sites in the dataset, a total of 8 patients were excluded from the per-patient analysis (3 with overall indeterminate pathology and 5 with no HRME images passing the automated quality control check).

The data from the remaining 229 cervical sites in 144 patients were included in the colposcopy and HRME performance analyses. Of these 229 cervical sites, 187 sites had a single HRME image collected and 42 sites had two HRME images collected. At sites where two HRME images were collected, the image with the highest (most abnormal) quantitative score was used. A histopathologic diagnosis of CIN2+ was diagnosed in 85 of the 229 cervical biopsy sites (37%) and in 50 of the 144 patients (35%). On a per-site basis, the distribution of histopathologic diagnoses was as follows: 87 benign, 57 CIN1, 39 CIN2, 39 CIN3, and 7 cancer. On a per-patient basis, the distribution of histopathologic diagnoses was as follows: 51 benign, 43 CIN1, 21 CIN2, 25 CIN3, and 4 cancer.

Examples of colposcopic and HRME images for sites in three different patients are shown in Fig. 3. The CIN1 cervical lesion in Fig. 3A was identified as negative by both colposcopy and HRME imaging. The CIN3 cervical lesion in Fig. 3B was identified as a high-grade lesion by both colposcopy and HRME imaging. However, the cervical lesion in Fig. 3C showed inflammation and was incorrectly identified as positive by both colposcopy and HRME, illustrating the possibility of a false positive result due to the confounding effects of inflammation. Inflammation accounted for a significant portion of the false positives for both colposcopy and HRME. A higher frequency of inflammation was observed among patients who were false positive by HRME than those who were false positive by colposcopy, though not statistically significant [per patient: 17/33 (52%) inflammation among HRME false positives; 14/38 (37%) inflammation among colposcopy false positives; $P = 0.24$; per site: 48/75 (64%) among HRME false positives; 35/72 (49%) among colposcopy false positives; $P = 0.07$].

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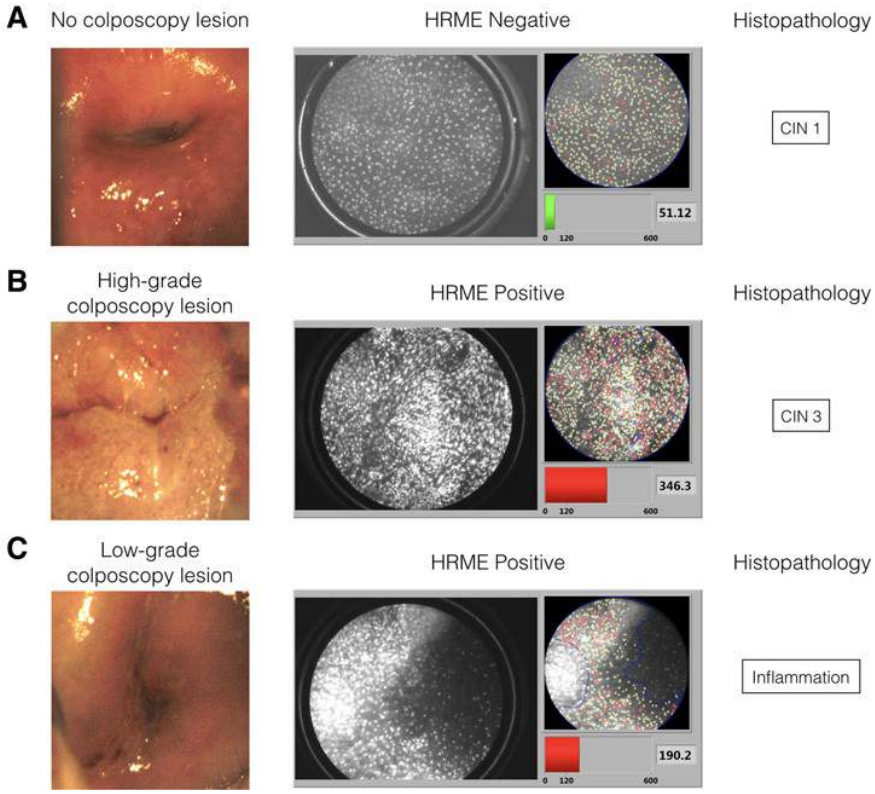

Figure 3.
Colposcopic and HRME images for three example patients. The histologic result is shown for reference. The HRME panels show a raw image of the nuclear morphology (left) and the automated analysis with quantitative interpretation (right). **A,** Both methods correctly identify the cervical site as benign. **B,** Both methods correctly identify the cervical site as a precancerous lesion. **C,** Both methods incorrectly indicated a precancerous lesion with the biopsy result showing inflammation. In the overlay, nuclei within normal limits for size and eccentricity are outlined in yellow; nuclei exceeding those limits are outlined in red. The parameter #abnormal nuclei/mm^2 is displayed at bottom right and in the bar graph relative to the threshold value (120). The bar is green if <120 (predicted nonneoplastic) or red if >120 (predicted neoplastic).

Figure 4 shows the secondary outcome results of the study: the sensitivity and specificity of colposcopy and *in vivo* microscopy for identification of CIN2+ with respect to histopathology. The sensitivity and specificity (with 95% binomial exact confidence intervals in parentheses) were as follows: on a per cervical lesion site (per-site) basis, low-grade colposcopy (threshold of low-grade or more abnormal) had a sensitivity of 94% (87%–98%) and a

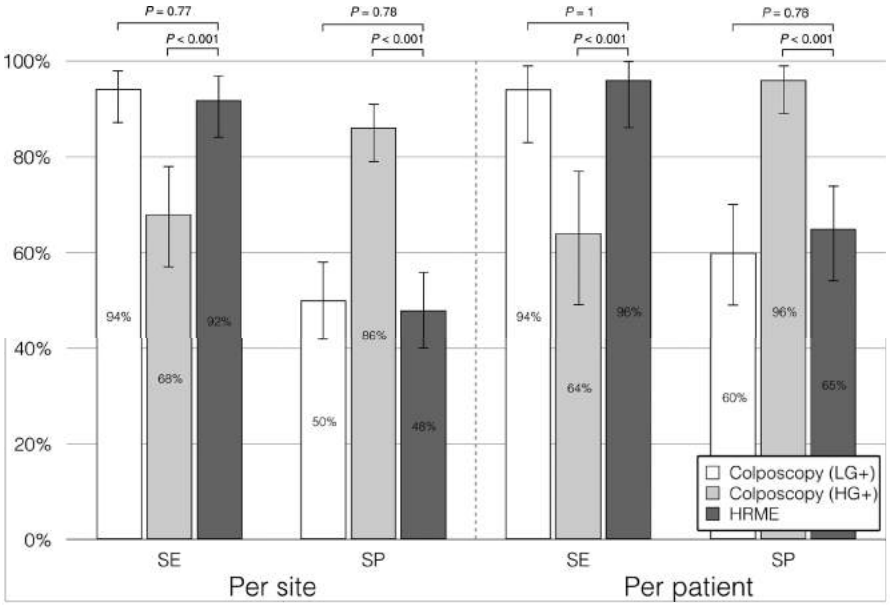

Figure 4.
Sensitivity (SE) and specificity (SP) of colposcopy and four-quadrant HRME for detecting CIN2+ per biopsy site and per patient. Colposcopy LG+ and HG+ indicate performance of colposcopy where low-grade and high-grade were used as the threshold for positivity, respectively. Error bars, binomial exact 95% CI. Significance testing was performed using McNemar's test.

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Table 3. *In vivo* microscopy results stratified by colposcopic impression and histologic diagnosis on a per-site and per-patient basis

		Per-site analysis ($n = 229$)		Per-patient analysis ($n = 144$)	
Colposcopic impression	Histologic diagnosis	No. of sites measured	No. (%) of sites HRME positive	No. of patients measured	No. (%) of patients HRME positive
Normal	Benign	51	24 (47)	36	9 (25)
	CIN 1	21	3 (14)	20	2 (10)
	CIN 2	2	2 (100)	1	1 (100)
	CIN 3	3	3 (100)	2	2 (100)
Abnormal	Benign	36	24 (67)	15	8 (53)
	CIN1	36	24 (67)	23	14 (61)
	CIN 2	37	33 (89)	20	19 (95)
	CIN 3	36	33 (92)	23	22 (96)
	AIS/invasive carcinoma	7	7 (100)	4	4 (100)

specificity of 50% (42%–58%), high-grade colposcopy (threshold of high-grade or more abnormal) had a sensitivity of 68% (57%–78%) and a specificity of 86% (79%–91%), and *in vivo* microscopy had a sensitivity of 92% (84%–97%) and specificity of 48% (40%–56%); on a per-patient basis, low-grade colposcopy had a sensitivity of 94% (83%–99%) and specificity of 60% (49%–70%), high-grade colposcopy had a sensitivity of 64% (49%–77%) and specificity of 96% (89%–99%), and *in vivo* microscopy had a sensitivity of 96% (86%–100%) and specificity of 65% (54%–74%). No significant statistical difference ($P > 0.05$) was found between low-grade colposcopy and *in vivo* microscopy in terms of sensitivity and specificity on a per-site or per-patient basis. High-grade colposcopy was found to be significantly more specific than *in vivo* microscopy ($P < 0.001$); however, *in vivo* microscopy was significantly more sensitive ($P < 0.001$), indicating a trade-off between sensitivity and specificity for high-grade versus low-grade colposcopy.

Table 3 shows the detailed results of *in vivo* microscopy for each subset of subjects stratified by colposcopic impression and histologic diagnosis. Of the 59 women in the study who had no colposcopic lesions, only three (5%) showed high-grade disease (CIN2+) by histopathology. HRME correctly classified all three (100%) of those patients as abnormal and correctly classified 45 of the remaining 56 (80%) as normal. In addition, among the 85 women who had lesions noted by colposcopy, 47 (55%) showed high-grade disease (CIN2+) by histopathology. HRME imaging correctly classified 45 (96%) of this subset of patients as abnormal and correctly classified 16 of the remaining 38 (42%) in the subset as normal.

Overall, HRME identified as positive 20 out of 21 (95%) of patients with CIN2, 24 out of 25 (96%) of patients with CIN3, and 4 out of 4 (100%) of patients with invasive cancer; and identified as negative 27 out of 43 (63%) of patients with CIN1 and 34 out of 51 (67%) of patients with benign histopathology (Table 3). Colposcopy identified as positive 20 out of 21 (95%) of patients with CIN2, 23 out of 25 (92%) of patients with CIN3, and 4 out of 4 (100%) of patients with invasive cancer; and identified as negative 20 out of 43 (47%) of patients with CIN1 and 36 out of 51 (71%) of patients with benign histopathology (Table 3).

Clinically normal sites where no biopsy was taken were excluded from the analysis because those sites lack a histopathologic gold standard. Only sites with a histopathologic gold standard were included in order to make the analysis as rigorous as possible. If clinically normal sites with no biopsy are included in the analysis, colposcopic examination must be used as the gold standard for those sites. This may be done, and it results in improved specificity for HRME imaging (HRME: 92% sensitivity, 81% specificity on a per-site basis).

Discussion

The primary findings of our study include a 37% relative and 23% absolute increase in the diagnostic completion follow-up rate of screen-positive women attending a mobile diagnostic van equipped with colposcopy and *in vivo* microscopy in their local community compared with those asked to travel to a central hospital for diagnosis. The diagnostic completion rate in the central hospital arm (considered baseline) was 64%, and the diagnostic completion rate in the mobile van arm was 87%. The absolute increase represents the difference between those percentages (87%–64% = 23%) and the relative increase represents the percentage increase relative to baseline (87%–64%)/64% = 37%, using unrounded values in the calculation. Furthermore, we found the combination of colposcopy and point-of-care *in vivo* microscopy to be feasible in a mobile van.

Current approaches for cervical cancer screening in high-income countries include Pap and/or HPV DNA testing (28). Patients with abnormal results undergo colposcopy with cervical biopsies and if clinically significant precursor lesions are identified, ablative (cryotherapy) or excisional procedures such as CKC or LEEP are performed. Although these screening and diagnosis algorithms are very effective, they are expensive and require high-level infrastructure and well-trained personnel. In addition, they require two or more separate patient visits with communication of test results between visits. These strategies therefore often fail in lower-resource settings such as underserved areas of Brazil and other LMICs where there is often a lack of trained personnel, infrastructure, and pathology services

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(3). Furthermore, geographical distances and cultural barriers result in many women with abnormal screening tests not receiving the recommended diagnostic and treatment procedures because they are unable to travel to central healthcare facilities for the multiple necessary follow-up visits (8). As a result, many women present with invasive disease often at an advanced stage when curative treatment is no longer achievable.

In the current study, HRME was found to have similar performance characteristics to colposcopy when using histopathologic diagnosis as the gold standard. On a per-patient basis, HRME had a sensitivity of 96% and specificity of 65% while colposcopy had a sensitivity of 94% and specificity of 60%. The analyses by cervical lesion site showed similar findings. In addition, HRME correctly identified 95% of the CIN2 lesions, 96% of the CIN3 lesions, and 100% of the invasive cancer lesions. These results are similar to previous study findings from this group evaluating the HRME device (12, 13). Pierce and colleagues (12) reported on 174 women in rural China who underwent cervical cancer screening with HPV testing, visual inspection with acetic acid (VIA), colposcopy and HRME imaging. Of the 69 women noted to have abnormalities on colposcopy, only 12 (17%) showed high-grade disease on biopsy. However, HRME imaging correctly classified all 12 high-grade areas (100%) as abnormal, and correctly classified 38 of the remaining 57 (67%) as normal. Furthermore, when patients were stratified based on a positive high-risk HPV DNA test, HRME imaging correctly identified 100% of the patients with CIN2+ (12). A subsequent report by Grant and colleagues (13) evaluated 59 women in Brazil undergoing colposcopy for abnormal screening Pap test results. In addition to colposcopy, all participants also underwent HRME imaging. Biopsies were obtained of abnormal areas and the histopathologic results were compared with the colposcopy and HRME findings. HRME was found to have a sensitivity of 92% and specificity of 77% compared with histopathologic diagnosis. The HRME device has also been shown to be effective as a diagnostic tool in both oral cancer and esophageal cancer screening (16–19).

This study was appropriately powered to detect a 30% difference in diagnostic completion rate. Because actual cluster size was smaller than the cluster size used in sample size calculations, the *post-hoc* statistical power was >95% over a very wide range of intracluster correlation coefficients.

When using the HRME, as with any point probe device, the clinician must choose where to place the probe. Colposcopy is one method to guide probe placement, but visual inspection can also be used. In this study, the HRME probe was placed (i) on colposcopically abnormal sites (guided by colposcopy) and (ii) on at least one site in each quadrant even if no colposcopic lesion was present (guided by visual examination).

The potential role of the HRME in the clinical setting depends on the other diagnostic resources that are available. In settings where colposcopy and histopathology are routinely available, the use of HRME in combination with colposcopy could potentially help guide the selection of biopsy sites, reduce the number of biopsies needed for diagnosis, and enable see-and-treat strategies. In settings where colposcopy and histopathology are not routinely available (and treatment decisions are often made on the basis of methods known to have poor specificity such as VIA), the use of HRME could potentially help improve specificity and reduce overtreatment.

One limitation of the HRME in this study was that images of acceptable quality were unable to be acquired for a small number of patients and cervical sites. The automated QC check failed in 6% of cervical sites imaged. Notably, over half of these QC failures occurred in a single very hot day early in the study in which the air conditioning unit within the mobile diagnostic van was not functioning, causing the HRME camera to overheat over the course of the day (a problem subsequently avoided simply by ensuring that the device was not left on continuously throughout the day). Another limitation of HRME is that it is not designed to sample the endocervix. Of the 45 patients that were both colposcopy negative and HRME negative, there were no CIN2+ cervical biopsies. However, 2 of these 45 women did have a positive ECC result (CIN3 in both cases) and were referred for treatment accordingly. Finally, HRME imaging requires the use of the fluorescent contrast agent proflavine. There were no adverse events related to proflavine exposure in this study; long-term follow-up studies of participants exposed to proflavine are ongoing.

One limitation of the study design is that the cost effectiveness and patient satisfaction of the mobile diagnostic van were not assessed. Additionally, all diagnostic and treatment procedures were performed by physicians fully trained as expert colposcopists, which would not be feasible in this setting outside of a research study.

In summary, our findings show that women referred to the mobile van equipped with colposcopy and *in vivo* microscopy were statistically significantly more likely to complete their diagnostic follow-up than women referred to a central hospital without any compromise in clinical care. *In vivo* microscopy provides an automated, point-of-care diagnostic imaging capability that can readily be implemented in a mobile van, with sensitivity and specificity similar to colposcopy.

Our results provide important information regarding the feasibility of this approach. Larger prospective studies evaluating HPV primary screening followed by HRME are ongoing in Brazil, El Salvador and in the Rio Grande Valley along the Texas–Mexico border. These studies will address some of the above limitations including the feasibility of performing HRME imaging in a variety of clinical settings and by nonspecialists including primary

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care physicians, nurse practitioners, midwives, and physician assistants.

Disclosure of Potential Conflicts of Interest.

R. Richards-Kortum has ownership interest (including patents) in IP licensed by Remicalm LLC. No potential conflicts of interest were disclosed by the other authors.

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