Clinical Performance of Human Papillomavirus Testing and Visual Inspection With Acetic Acid in Primary, Combination, and Sequential Cervical Cancer Screening in China

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Background: World Health Organization guidelines recommend screening with human papillomavirus (HPV) testing followed by either treatment of all HPV-positives, or by visual inspection (VIA) for triage to treatment, citing insufficient evidence to recommend either strategy over the other.

Methods: We assessed VIA and HPV testing individually, in combination (HPV-VIA cotesting), and as triage models. Three thousand women were screened in Inner Mongolia, China, concurrently with HPV testing and VIA in a real population setting. Screen-positive women underwent colposcopy, and biopsy, if indicated. Accuracy of screening algorithms for cervical intraepithelial neoplasia grade 2 or higher (CIN-2+) was calculated after controlling for verification bias. HPV testing followed by VIA triage for CIN-2+ detection was compared with Hybrid Capture 2 viral loads triage, measured in relative light units/cutoff.

Results: CIN-2+ prevalence was 1.0%. Corrected sensitivity, false negative rate, and specificity for CIN-2+, respectively, for primary HPV testing were 89.7%, 10.3%, and 83.3%; 44.8%, 55.2%, and 92·3% for VIA; 93.1%, 6.9%, and 80.2% for HPV-VIA cotesting; and 41.4%, 58.6, and 95.4% for HPV with VIA triage scenarios. Using relative light units/cutoff of 5 or greater to triage HPV-positive women had twice the sensitivity as VIA triage, with comparable specificity for CIN-2+.

Conclusions: When VIA performs relatively poorly and HPV testing is available, adding VIA to sequential (ie, HPV followed by VIA triage) or primary (HPV-VIA cotesting) screening does not significantly improve CIN-2+ detection beyond primary HPV screening alone. Sequential screening (ie, HPV followed by VIA triage) reduces sensitivity too low for population-based screening programs. The HPV viral loads could offer an alternative low-resource country triage strategy.

Low-resource countries (LRC) carry over 85% of cervical cancer disease burden.[1,2] In China, cervical cancer was the second most common cancer among 30- to 40-year-old women and caused 30,500 deaths in 2015.[3] Cytology-based screening programs have reduced cervical cancer mortality rates in developed countries, but are difficult to reliably implement in LRC that lack trained cytopathologists, pathology laboratories, or patient follow-up infrastructure.[4,5]

Visual inspection with diluted acetic acid (VIA), and human papillomavirus (HPV) testing are alternative LRC screening strategies.[5] Visual inspection with diluted acetic acid is inexpensive and can be performed by lay providers with training, and VIA-positive women can be treated in a single visit, reducing loss to follow-up.[6] However, VIA performance varies with provider experience, with sensitivities for detecting histologically confirmed high-grade precancerous lesions (cervical intraepithelial neoplasia grade 2 or worse; CIN-2+) ranging from 41% to 92% and specificities

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ranging from 49% to 98% between studies.[7,8] The VIA results are less reliable in postmenopausal women.[9]

In terms of primary screening for CIN-2+, HPV testing reduces cervical cancer incidence and mortality more than cytology or VIA with one screen.[10,11] Primary HPV testing has a sensitivity of 89.7% (range, 86.4% to 93.9%) and specificity of 88.2% (range, from 86.2% to 90.1%) for CIN-2+ detection.[12] HPV-positive women with transient high-risk (hr) HPV infections, however, are at risk for overtreatment, and so triaging only hrHPV-positive women with progressive infections for treatment would be ideal.[13]

In 2013, the World Health Organization (WHO) made recommendations for HPV testing and VIA screening algorithms for screen-and-treat programs in LRC conditionally, citing that low-quality or very-low-quality evidence was available on their accuracy: (i) recommendation 2—Screen with HPV testing over screen with VIA, and treat; (ii) recommendation 6—screen with HPV testing followed by VIA triage, or screen with HPV testing, and treat; (iii) recommendation 7—screen with HPV testing followed by VIA triage, over screen with VIA, and treat.[14]

A demonstration project was conducted in 2009 in the Inner Mongolia Autonomous Region, 4 years before the 2013 WHO recommendations on cervical cancer screening. Women were screened with both VIA and HPV DNA testing. Screen-positive women were referred to colposcopy, with biopsy taken if indicated.

We aimed to evaluate the recommended WHO VIA and with HPV testing cervical cancer screening algorithms in a real-population setting, to determine: (i) the clinical performance of primary HPV testing alone, and VIA testing alone for CIN-2+ detection (WHO recommendation 2); (ii) whether adding VIA to primary HPV testing (HPV-VIA cotesting) increases screening performance compared with primary HPV testing alone; and (iii) how to best triage HPV-positive women in low-resources settings (WHO recommendations 6 and 7).

MATERIALS AND METHODS

Inner Mongolia Autonomous Region People's Hospital clinicians conducted cervical cancer screening demonstration projects from April to August 2009 in three rural regions of Inner Mongolia, China: Ordos, Tongliao, and Xing'an. Women were eligible to participate if they were 18 years or older, married, sexually active, not pregnant, had intact uteri, and no history of CIN-2+ disease or previous pelvic radiation, and had not underwent cervical cancer screening within the last 5 years. Women who participated signed informed study consent forms, and were screened for cervical cancer concurrently with HPV DNA testing (Hybrid Capture 2 [HC2] assay; QIAGEN, Gaithersburg, MD) and with VIA.

Institutional review boards at the Inner Mongolia Autonomous Region People's Hospital, Chinese Hospital and Institute at Chinese Academy of Medical Sciences, and University of North Carolina at Chapel Hill approved this study.

Quality Assurance of Study

VIA Screening

An experienced physician from Beijing, with over 30 years of experience conducting VIA, recruited and trained seven local clinical providers from Inner Mongolia Autonomous Region People's Hospital. Of these 7 clinical providers, 4 had completed medical school and 3 nursing school. Each local provider was required to have at least 5 years of prior VIA screening experience and complete a one-day training program.

At commencement of on-site screening, the experienced physician provided field-based instruction by first conducting VIA and HPV DNA testing while clinical providers observed and then directly supervised each provider as they performed the screenings with feedback, if necessary. As the local providers performed screenings throughout the project duration, the experienced physician was present in the room for direct oversight and questions.

Collection of Sociodemographic Data

Trained health workers at each site explained the study, screening process, potential risks and benefits, and answered questions from participants. After completion of registration, informed consent, and a questionnaire, women entered the examination room for routine gynecological examinations followed by screening with VIA and HPV DNA testing.

Clinical Supervision

The experienced physician oversaw all screenings and colposcopy examinations conducted by two to three local clinicians at all sites throughout the entire demonstration project.

Screening Tests

Visual Inspection

Local Chinese physicians performed visual inspection by applying 5% acetic acid to the cervix, waiting 1 minute, and then inspecting the cervix for abnormal changes under a 100-Watt incandescent light source. VIA positivity was characterized by well-defined, opaque acetowhite lesions in the cervical transformation zone.

HPV Testing

Laboratory technicians, blinded to VIA clinical outcomes, conducted HPV-DNA tests using the HC2 test on exfoliated cervical samples within 2 weeks of specimen collection. HC2 detects DNA of 13 carcinogenic hrHPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Cervical samples were deemed HPV-positive according to manufacturer instructions based on relative light unit to positive control ratio (RLU/CO) being 1.0 or higher (approximately equal to 1.0 pg DNA/mL). Semi-quantitative estimates of the viral load of HC2-positive specimens were made according to RLU/CO values.

Cervical Biopsy

Women positive for either HPV or VIA screening tests underwent colposcopy examination. Women with an abnormal VIA were examined with colposcopy during the same visit. HPV-positive women were called back for colposcopy within 2 weeks of the initial screening. Directed biopsies using a 2-mm bronchoscopy biopsy instrument were taken from all visible cervical lesions. When the four-quadrant punch biopsy method was indicated,[15] biopsies were taken at positions of 2 o'clock, 4 o'clock, 8 o'clock, and 10 o'clock. If the colposcopy examination was unsatisfactory, ectocervical curettage samples were also taken.

Verification of Cervical Disease Status

The gold standard of disease verification was the histologically confirmed biopsy pathology result taken after colposcopy. Chinese Hospital and Institute at Chinese Academy of Medical Sciences pathologists examined all cervical biopsy samples. Histology diagnoses were categorized as negative, CIN grades 1, 2, 3, carcinoma in situ, squamous cell carcinoma, adenocarcinoma in situ, or adenocarcinoma according to the FIGO staging system. A diagnosis of CIN-2+ was the primary clinical outcome, and included CIN-2, CIN-3, carcinoma in situ, squamous cell carcinoma, adenocarcinoma in situ, or adenocarcinoma diagnoses. Technicians

and pathologists who reviewed and diagnosed slides were blinded to primary screening results.

Study Population for Statistical Analyses

Women with unsatisfactory HC2 testing or VIA results (N = 42) or those lost to colposcopy follow-up (N = 16) were excluded from analyses, leaving 2,942 women with satisfactory HC2, VIA, and colposcopy results. Women younger than 30 (no cases of CIN-2+ detected in this age group) or if age was unknown (N = 274) were further excluded, leaving 2668 women in the final population analysis (Appendix 1).

Statistical Analysis

Estimated prevalences of hrHPV, abnormal VIA, CIN and cervical cancer were stratified by age group (30–39 years, 40–49 years, and ≥50 years), with differences calculated using *P* values. Women were classified as normal if both HPV and VIA screening tests were negative or they had at least one positive screening test but no evidence of cervical precancerous lesions on histology. Women with dually negative VIA and HPV results did not have cervical biopsies taken, as pathology would likely not show high-grade cervical disease.[15] Prevalence of normal, CIN-1, CIN-2, and CIN-3 was calculated according to the total number of women screened for each age group.

Estimated CIN-2+ cases that might have been missed in each age group were calculated using the probability of CIN-2+ generated from our previously well-described study (Shanxi Province Cervical Cancer Screening Study),[15] where all participating women received colposcopy-directed biopsies regardless of test positivity. "Corrected" refers to correction for verification bias with regards to histological endpoint, while "uncorrected" does not correct for verification bias.[16] Corrected sensitivity, specificity, false negative rate (FNR), positive predictive value (PPV), and negative predictive value (NPV) with corresponding 95% confidence intervals (CIs) for the various screening algorithms, HC2 viral loads, and triage methods were calculated[16] for the clinical endpoint of CIN-2+. Area under the curve (AUC) of the receiver operating characteristics (ROC) and associated 95% CIs were calculated for the various screening algorithms, HC2 viral loads, and triage methods.

McNemar's χ^2 test for paired comparison and the χ^2 test were used to detect statistically significant differences in the clinical performance of the different screening algorithms, HC2 viral loads, and triage methods. The z-test was used to evaluate differences in AUC between screening tests with a significance level of 0.05. Statistical analyses were performed using SAS 9·2.

RESULTS

Among 2,668 screened women, the mean age was 41.6 years (range, 30–59 years). The prevalence of HPV infections in the women aged 18 to 30 years was 20.7% (29/140). For women 30 years or older, under one quarter were HPV-positive (17.5%; N = 467), 8.1% had an abnormal VIA result (N = 216), 98.1% had normal pathology (N = 2616), 0.9% had CIN-1 (N = 25), and 1.0% had CIN-2+ (N = 27) (Table 1). Most participating women (98.1%, N = 2,616) were considered to have normal pathology; this figure includes 2,114 women with negative HPV and negative VIA screening results who were assumed to have normal pathology and thus not referred to colposcopy, and 502 women with negative biopsies upon histology. There were no significant differences in the prevalence of HC2 HPV test positivity and the distribution of histological grades of normal, CIN-1, CIN-2, or CIN-3 to cervical cancer, stratified by age group ($P > 0{\cdot}05$), while the prevalence of abnormal VIA decreased with increasing age group ($P = 0.012$).

HPV Testing and VIA Screening Algorithms for CIN-2+

When comparing primary screening results, HPV DNA testing detected twice as many CIN-2+ cases (N = 26, 0.97%) as VIA screening (N = 13, 0.49%) (Table 2). Using HPV-VIA cotesting as primary screening resulted in only 1 additional CIN-2+ case detected (*N* = 27, 1.01%) compared with primary HPV testing alone. Triage screening algorithms, both HPV testing followed by VIA triage, and VIA followed by HPV triage, detected only 12 cases of CIN-2+ (0.45%).

Comparing the different screening algorithms using ROC curves resulted in corrected AUC being highest for both primary HPV testing (0.87) and HPV-VIA cotesting (0.87), and lowest for both triage models of HPV testing followed by VIA triage (0.68), and VIA followed by HPV triage (0.68) (Fig. 1).

Uncorrected sensitivity ranged from a high of 100% (95% CI, 87.5–100.0) for HPV-VIA cotesting, to a low of 44.4% (95% CI, 27.6–62.7) for both triage models. Of note, primary HPV testing had an overall uncorrected sensitivity of 96·3% (95% CI, 81.7–99.3).

A similar pattern was seen for corrected sensitivity, ranging from a high of 93·1% (95% CI, 78.0–98.1) for HPV-VIA cotesting, to a low of 41.4% (95% CI, 25.5–59.3) for both triage models.

The corrected FNR was highest for the triage models (58.6%; 95% CI, 40.7–74.5) to lowest for HPV-VIA cotesting (6.9%; 95% CI, 1.9–2.2). The HPV primary testing had an FNR of 10.3% (95% CI, 3.6–26.4).

TABLE 1. Prevalence of HC2 HPV Test Positivity, Abnormal VIA, and Cervical Intraepithelial Neoplasia, Stratified by Age Group

								Prevalence of Cervical Precursor Lesions and Cervical Cancer							
	Women Screened	HPV+ by HC2 test		Abnormal VIA		Women not Referred to Colposcopy*		Normal†		CIN1		CIN2		CIN3+‡	
Age, y	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%
30–39	1093	195	17.8	108	9.9	857	78.4	1070	97.9	12	0.4	7	0.6	4	0.4
40–49	1100	200	18.2	80	7.3	864	78.5	1076	98.7	9	0.3	7	0.6	8	0.7
≥50	475	72	15.2	28	5.9	393	82.7	470	98.9	4	0.14	1	0.2	0	0.0
Total	2668	467	17.5	216	8.1	2114	79.2	2616	98.1	25	0.9	15	0.6	12	0.4
P		0.325	0.012			0.637									

*Women were referred to colposcopy only in the case of positive VIA, or positive HPV HC2 test results, or being opportunistic to undergo colposcopy (N = 5).

†502 participants that had a normal histology result, and 2,114 participants that had negative HPV and VIA results.

‡3 cases of invasive cervical cancer.

TABLE 2. Clinical Performance of Cervical Cancer Screening Algorithms with HPV Testing and VIA, for CIN2+ Detection*Among 2,668 Women

Screening strategy category	CIN2+ cases (N)	CIN2+ Rate %	Corrected AUC, % (95% CI)	Uncorrected Sensitivity,% (95% CI)	Corrected Sensitivity,% (95% CI)	Corrected FNR, % (95% CI)	Corrected Specificity,% (95% CI)	Corrected PPV,% (95% CI)	Corrected NPV,% (95% CI)	Referral Cases (N)	Referral Rate,% (95% CI)
HPV primary testing	26	0.97	0.87 (0.80–0.93)	96.3 (81.7–99.3)	89.7 (73.6–96.4)	10.3 (3.6–26.4)	83.3 (81.8–84.7)	5.6 (3.8–8)	99.9 (99.6–100)	467	17.5 (16.1–19)
VIA abnormal	13	0.49	0.69 (0.57–0.80)	48.1 (30.7–66.0)	44.8 (28.4–62.5)	55.2 (37.5–71.6)	92.3 (91.2–93.3)	6.0 (3.6–10.0)	99.3 (98.9–99.6)	216	8.1 (7.1–9.2)
HPV testing and VIA (co-testing)	27	1.01	0.87 (0.81–0.92)	100.0 (87.5–100.0)	93.1 (78.0–98.1)	6.9 (1.9–22)	80.2 (78.7–81.7)	4.9 (3.4–7.1)	99.9 (99.7–100)	549	20.6 (19.1–22.2)
HPV+ then triage by VIA	12	0.45	0.68 (0.57–0.80)	44.4 (27.6–62.7)	41.4 (25.5–59.3)	58.6 (40.7–74.5)	95.4 (94.5–96.1)	9.0 (5.2–15.0)	99.3 (98.9–99.6)	134	5.0 (4.3–5.9)
VIA+ then triage by HPV	12	0.45	0.68 (0.57–0.80)	44.4 (27.6–62.7)	41.4 (25.5–59.3)	58.6 (40.7–74.5)	95.4 (94.5–96.1)	9.0 (5.2–15.0)	99.3 (98.9–99.6)	134	5.0 (4.3–5.9)

*CIN2+ cases were compared with women without CIN2 + .

Referral cases, absolute number of cases screened positive referred for colposcopy; referral rate, referral rate for colposcopy. Corrected AUC, FNR, sensitivity, specificity, PPV, and NPV figures correct for verification bias.

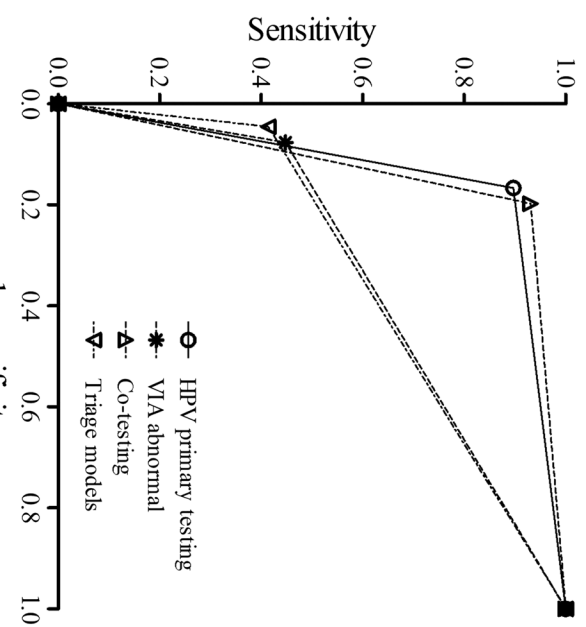

Figure 1. ROC of cervical cancer screening algorithms with HPV testing and VIA, for CIN2+ detection among 2688 women.

Corrected specificities were relatively high for all five screening algorithms, ranging from 80·2% (95% CI, 78.7–81.7) for primary HPV testing, to 95.4% (95% CI, 94.5–96.1) for both triage models.

Corrected PPV ranged from 4.9% (95% CI, 3.4–7.1) for HPV-VIA cotesting, to 9.0% (95% CI, 5.2–15.0) for both triage models. Corrected NPV were high for all screening algorithms (>99%).

The number of cases referred for further screening with colposcopy, a measure of clinical resources usage, ranged noticeably across the screening algorithms. The fewest number of women referred was with the triage models (N = 134, 5.0%), and greatest with HPV-VIA cotesting (N = 549 cases, 20.6%).

HPV Viral Load as a Possible Triage Option

Triaging HPV-positive women avoids overtreatment. Regarding the performance of HPV viral load triage, increasing the RLU/CO of HPV testing from 1.0 to 100 (pg of hrHPV DNA) decreased the number of detected CIN-2+ cases, the test sensitivity, and colposcopy referral rate, and increased the test FNR and specificity (Table 3). As viral load cutoff increased, AUC of ROCs decreased (Fig. 2).

Among HPV-positive women, defined as HC2 test RLU/CO ≥ 1 (N = 467), further triage by HPV viral load detected CIN-2+ from a high of 22 cases at RLU/CO ≥ 2 to a low of 8 cases at RLU/CO ≥ 100 (Table 4). Regarding HPV testing followed by HPV viral load triage, increasing the RLU/CO cutoff decreased screening sensitivity, increased the FNR and specificity, and decreased the colposcopy referral rate. Among the 467 HPV-positive women, VIA triage had a sensitivity of 46.2%, FNR of 53.8%, specificity of 72·3%, and referred 134 women to colposcopy. Comparably, HPV testing followed by RLU/CO ≥5 triage had a sensitivity of 84·6%, FNR of 15.4%, specificity of 62.4%, and referred 188 women. Compared with HPV testing followed by VIA triage, HPV testing followed by RLU/CO ≥5 triage had improved clinical performance, with increased sensitivity by 38.4% ($P = 0.013$), AUC by 0.1% ($P = 0·085$) and a reasonable decrease in specificity by 9.9% ($P = 0.002$). All modalities had similar PPVs (ranging from 9.6% to 12.5%) and a NPV > 95%.

TABLE 3. Clinical Performance of HPV Testing, Stratified by HPV Viral Load, CIN2+ Detection Among 2,668 Women

Cutoff Ratio (RLU/CO)	CIN2+ Cases (N)	Uncorrected Sensitivity, % (95% CI)	Corrected Sensitivity, % (95% CI)	Corrected FNR, % (95% CI)	Corrected Specificity, % (95% CI)	Corrected PPV, % (95% CI)	Corrected NPV, % (95% CI)	Referral Cases (N)	Referral Rate, % (95% CI)	Corrected AUC, % (95% CI)
1.0	26	96.3 (81.7–99.3)	89.7 (73.6–96.4)	10.3 (3.6–26.4)	83.3 (81.8–84.7)	5.6 (3.8–8)	99.9 (99.6–100)	467	17.5 (16.1–19)	0.9 (0.85–0.94)
2.0	22	81.5 (63.3–91.8)	75.9 (57.9–87.8)	24.1 (12.2–42.1)	92.2 (91.1–93.2)	9.6 (6.5–14.2)	99.7 (99.4–99.9)	228	8.5 (7.5–9.7)	0.87 (0.78–0.95)
3.0	22	81.5 (63.3–91.8)	75.9 (57.9–87.8)	24.1 (12.2–42.1)	93.3 (92.3–94.2)	10.5 (7.1–15.4)	99.7 (99.4–99.9)	209	7.8 (6.9–8.9)	0.87 (0.79–0.96)
4.0	22	81.5 (63.3–91.8)	75.9 (57.9–87.8)	24.1 (12.2–42.1)	93.3 (92.3–94.2)	11.1 (7.4–16.2)	99.7 (99.4–99.9)	199	7.5 (6.5–8.5)	0.87 (0.79–0.96)
5.0	22	81.5 (63.3–91.8)	75.9 (57.9–87.8)	24.1 (12.2–42.1)	93.7 (92.7–94.6)	11.7 (7.9–17.1)	99.7 (99.4–99.9)	188	7.0 (6.1–8.1)	0.85 (0.76–0.94)
10.0	16	59.3 (40.7–75.5)	55.2 (37.5–71.6)	44.8 (28.4–62.5)	94.8 (93.9–95.6)	10.5 (6.6–16.4)	99.5 (99.1–99.7)	152	5.7 (4.9–6.6)	0.77 (0.66–0.88)
20.0	15	55.6 (37.3–72.4)	51.7 (34.4–68.6)	48.3 (31.4–65.6)	96.1 (95.3–96.8)	12.7 (7.9–19.9)	99.5 (99.1–99.7)	118	4.4 (3.7–5.3)	0.76 (0.64–0.88)
100.0	8	29.6 (15.9–48.5)	27.6 (14.7–45.7)	72.4 (54.3–85.3)	97.9 (97.3–98.4)	12.5 (6.5–22.8)	99.2 (98.8–99.5)	64	2.4 (1.9–3.1)	0.64 (0.51–0.76)

Corrected AUC, FNR, sensitivity, specificity, PPV, and NPV figures correct for verification bias.

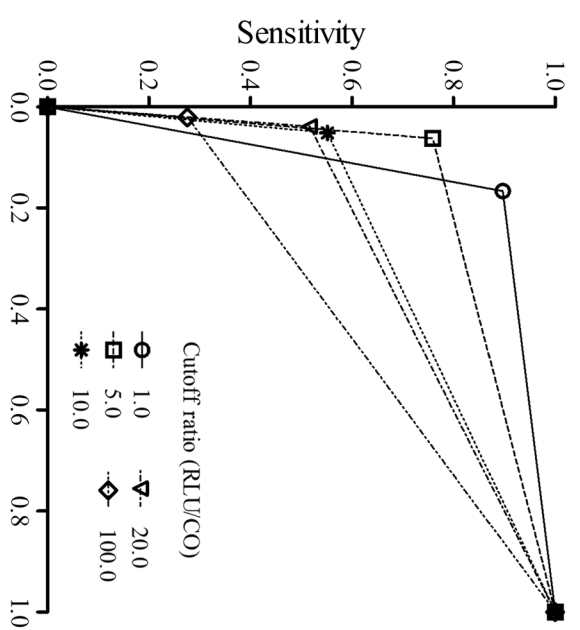

Figure 2. ROC of HPV viral load levels determined by HC2 HPV test, for CIN2+ detection among 2,688 women.

DISCUSSION

Our study evaluated the performance of VIA and HPV testing alone, together, and as triage models for CIN-2+ detection in over 2,500 screened women to inform WHO recommendations 2, 6, and 7 for screen-and-treat programs in LRC. Our data suggest that, when VIA has a relatively low sensitivity, screening with primary HPV testing alone should be used instead of HPV followed by VIA triage (informs recommendation 6: Screen with HPV testing followed by VIA triage, or screen with HPV testing, and treat). Human papillomavirus followed by VIA triage performed comparable to primary VIA screening alone (supports recommendation 7: Screen with HPV testing followed by VIA triage, over screen with VIA, and treat). Primary HPV testing was more accurate than primary VIA screening in our study (supports recommendation 2: Screen with HPV testing over screen with VIA, and treat). Our data do not support using VIA in screening algorithms in settings where HPV testing is available. Triage of HPV-positive women by HPV viral load rather than VIA could be more suitable in LRC.

Prevalence of CIN-2+ in Inner Mongolia (1·0%) is comparable to CIN-2+ prevalence in China (<2%).[17–19] Primary HPV testing was more sensitive (26 CIN-2+ cases) compared with primary VIA screening (13 CIN-2+ cases), and had a lower FNR, which supports recommendation 2, using HPV testing over VIA for primary screening. HPV-VIA cotesting had comparable sensitivity, specificity, and FNR to primary HPV testing alone. The addition of VIA to HPV testing resulted in 82 more colposcopy referrals with only one additional CIN-2+ case detected. A demonstration project in India reached similar results,[20] suggesting that adding VIA to HPV testing leads to a much higher colposcopy referral rate without a concomitant increase in the rate of high-grade CIN detection.

World Health Organization recommends either a sequential screening strategy of HPV testing followed by VIA triage or primary HPV testing (recommendation 6), and HPV testing followed by VIA triage over primary VIA screening alone (recommendation 7). In our study, triaging HPV test-positive women with VIA drastically decreased sensitivity by 51.9%, compared with primary HPV testing (N = 12 vs. 26), with a lower improvement in specificity by 12·1%. This large drop in sensitivity renders the screening algorithm

TABLE 4. Comparison of Triage Methods Following Positive HPV Tests in Screening for CIN2+ Among 467 HC2 HPV Positive Participants

Triage Method*	CIN2+ Cases (N)	Sensitivity, % (95% CI)	FNR % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Referral cases (N)	Referral Rate, % (95% CI)	AUC, % (95% CI)
RLU/CO ≥ 2	22	84.6 (66.5–93.8)	15.4 (6.2–33.5)	53.3 (48.6–57.9)	9.6 (6.5–14.2)	98.3 (95.8–99.3)	228	48.8 (44.3–53.3)	0.69 (0.60–0.78)
RLU/CO ≥ 5	22	84.6 (66.5–93.8)	15.4 (6.2–33.5)	62.4 (57.8–66.8)	11.7 (7.9–17.1)	98.6 (96.4–99.4)	188	40.3 (35.9–44.8)	0.74 (0.65–0.82)
RLU/CO ≥ 10	16	61.5 (42.5–77.6)	38.5 (22.4–57.5)	69.2 (64.7–73.3)	10.6 (6.6–16.5)	96.8 (94.3–98.3)	152	32.5 (28.5–36.90	0.65 (0.54–0.77)
RLU/CO ≥ 100	8	30.8 (16.5–50)	69.2 (50–83.5)	87.3 (83.9–90.1)	12.5 (6.5–22.8)	95.5 (93.1–97.2)	64	13.7 (10.9–17.1)	0.59 (0.49–0.71)
Abnormal VIA	12	46.2 (28.8–64.5)	53.8 (35.5–71.2)	72.3 (68.0–76.3)	9.0 (5.2–15)	95.8 (93.1–97.5)	134	28.7 (24.8–33.0)	0.59 (0.48–0.71)

*Triage in 467 women who had positive HC2 test, defined as RLU/CO ≥ 1.

of HPV testing followed by VIA triage unsuitable for a mass-screening program, which informs recommendation 6 that primary HPV testing alone should be used instead of HPV followed by VIA triage for screening. Similarly, a study in Cameroon reported a 66% decrease in sensitivity with HPV testing followed by VIA triage compared with self-HPV testing for CIN-2+ detection[21] and a study in India reported a 29·6% drop in sensitivity for CIN-3+ detection without concomitant increase in specificity.[22]

Our study findings support recommendation 7 in that the sensitivity, FNR, specificity and colposcopy referral rate of HPV followed by VIA triage was comparable to that of primary VIA screening alone. Regarding WHO recommendations 6 (either a sequential screening strategy of HPV testing followed by VIA triage or primary HPV testing) and 7 (HPV testing followed by VIA triage over primary VIA screening alone), HPV testing followed by VIA triage in our study did not offer a better balance between specificity and sensitivity as compared with primary HPV or VIA testing. Primary HPV testing had the best balance between screening performances with clinical resource usage (measured by colposcopy referrals) among the assessed algorithms.

Compared with primary VIA testing, the sensitivity of VIA followed by HPV triage appeared too low for a mass screening program to offset its gain in specificity for CIN-2+ detection. Contrary to our conclusions, other studies found VIA followed by HPV triage to perform well.[22,23] Superior results of VIA followed by HPV triage could be explained by VIA's relatively greater accuracy in those studies (67–82.3% for CIN-2+ sensitivity and 66–93.2% for specificity). In this study, primary VIA screening had a high FNR (55.2%), and a corrected sensitivity of 44.8%, which is lower that of the previous Shanxi Province Cervical Cancer Screening Study I study in China (71%),[15] but comparable to the average sensitivity of VIA in controlled study settings (50%), and to a pooled analysis of studies in China (54·6%).[24,25]

The VIA provider experience likely explains variations in VIA test sensitivity across studies.[8] It is possible that the higher sensitivity of VIA observed in the SPOOCS-1 trial as compared with our current findings could have been due to differences in provider training on VIA techniques, and this requires further investigation. Our study included 4 clinicians who completed medical training and three who completed nursing training. The strength of our study is that it captures VIA performance by providers in real-world, resource-limited settings. The difference in VIA performance in a real population-based screening setting may explain why VIA followed by HPV triage, if VIA is performed relatively poorly, would not be a suitable mass screening strategy for those areas.

Depending on an area's HPV prevalence, screening programs can decide if triage screens of HPV-positive women is worthwhile for a relative loss in sensitivity for CIN-2+ with gain in specificity. An efficient triage strategy is crucial in areas of high HPV prevalence, such as Inner Mongolia (17·5%). In this study, where VIA sensitivity is relatively low, the triage of HPV-positive women by VIA dramatically reduced the efficacy of a population-based screening strategy (decreased sensitivity by 51.9% and increased FNR by 48.3%, with only a 12.1% gain in specificity). Yet triage of HPV-positive women is needed to reduce unnecessary procedures and further and more invasive testing.

Growing evidence shows that measuring baseline hrHPV viral load predicts cervical cancer risk,[26,27] although data has been inconsistent across studies.[28,29] In our study, increasing the viral load cutoff of CIN-2+ positivity resulted in increased specificity and lower colposcopy referral rates, although decreased sensitivity and increased FNR. Depending on screening program needs, hrHPV viral load may be considered for triage of HPV-positive women, depending on how much loss in sensitivity for relative gain in specificity is acceptable. With loss of sensitivity, the detection rate

of CIN2+ will be lower, although test specificity for CIN2+ detection would be relatively higher, which would result in a lower the colposcopy referral rate. Individual programs would have to consider the most suitable cutoff for triage based on regional needs. In our present study, we used a semi-quantitative measure of HPV viral load with HC2 RLU/CO cutoffs. Depending on screening program needs, semi-quantitative estimates of hrHPV viral load based on RLU/COs from the HC2 test results, may be considered for triage of HPV-positive women, depending on how much loss in sensitivity for a relative gain in specificity is acceptable. For example, among HPV-positive women (RLU/CO ≥ 1) in our study, triage using a viral load cutoff of RLU ≥ 5 struck a relatively acceptable balance between decrease in sensitivity with increase in specificity for CIN-2+, and lower colposcopy referral rates with increased FNR. It should be noted, however, that a more accurate, quantitative ascertainment of HPV viral load using highly sensitive PCR ascertainment would have notable cost and operation hurdles for successful implementation in low-resource countries.

This study presents realistic performance of HPV and VIA screening tests in a resource-limited setting by LRC providers of varying levels of expertise. Most participants undergoing colposcopies had biopsies taken, allowing for precise disease verification, and thus accurate estimates of the screening methods' performances. The study had high-quality disease verification: both HPV and VIA screening tests had to be negative for the classification of negative disease and highly trained pathologists read all histological slides.

Among study weaknesses, screened-negative women did not have the diagnostic reference standard, as that is neither ethical nor feasible in a demonstration project. Alternatively, we adjusted for verification bias using standard calculations,[16] although resulting sensitivity measures may be overestimated and FNR measures may be underestimated. Performances of triage models were simulated, and these screening algorithms should be carried out in a real population setting to further assess different triage strategies following an HPV-positive test using a randomized control study design. Accuracy of screening algorithms presented should also be conducted with a greater number of CIN-2+ cases to increase statistical power. We found that the prevalence of HPV and of CIN2+ histology did not appear to differ when stratifying by age groups (30–39 years, 40–49 years, >50 years) which was unexpected, and inconsistent with several studies which has often shown prevalence increase or inconsistent across age.[30,31]

In areas where provider performance of VIA is poor, and valid HPV testing is available, adding VIA to primary (cotesting) or sequential (ie, HPV followed by VIA triage) screening algorithms do not significantly improve CIN-2+ detection. Good-quality VIA may improve detection but poorly conducted VIA may not, as VIA is a highly variable test, whereas HPV testing is less variable and highly reproducible. The VIA program performance has been shown to increase notably when cervicography is used systematically as a quality assurance measure.[32] Our data support using primary HPV testing over primary VIA screening (recommendation 2) in LRC and suggests using primary HPV testing over HPV testing followed by VIA triage where VIA performs relatively poorly. Findings support WHO recommendation 2—screen with HPV testing over screen with VIA, and treat; recommendation 6—screen with HPV testing followed by VIA triage, or screen with HPV testing, and treat; and recommendation 7—screen with HPV testing followed by VIA triage, over screen with VIA, and treat. Further studies are needed, but triage of HPV test-positive women by viral load could present a potentially effective triage strategy for mass-screening programs in LRC without relying on provider experience.

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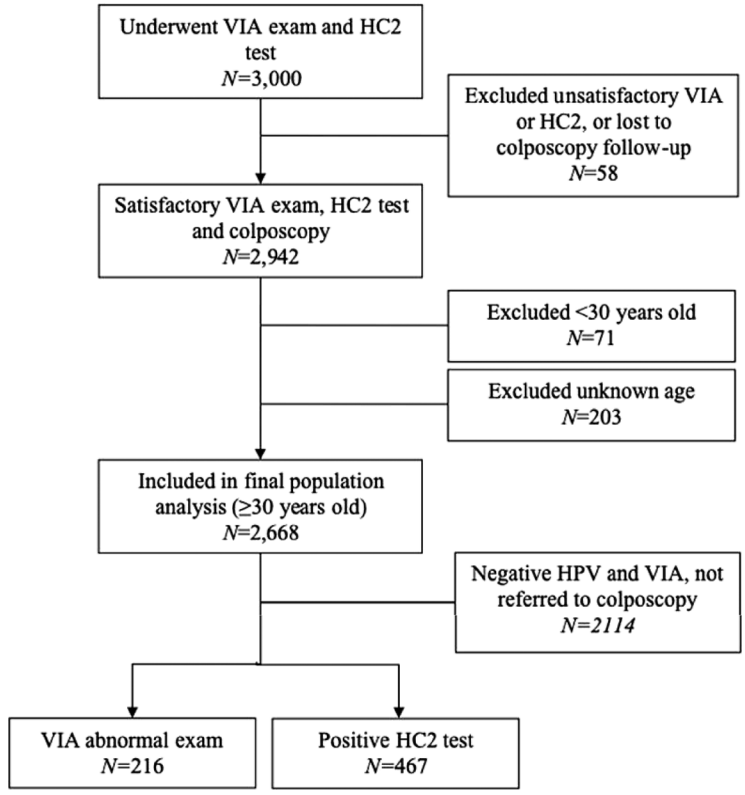
Appendix 1. Flowchart of study participants.



VIA=Visual inspection with acetic acid; HC2=Hybrid Capture® 2. A total of 216 women screened positive by VIA; 467 women by HC2; 134 women had both VIA and HC2 DNA testing positivity.