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Lower cost strategies for triage of human papillomavirus DNA-positive women

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Using human papillomavirus (HPV) testing for cervical cancer screening in lower-resource settings (LRS) will result in a significant number of screen-positive women. This analysis compares different triage strategies for detecting cervical precancer and cancer among HPV-positive women in LRS. This was a population-based study of women aged 25–65 years living in China ($n = 7,541$). Each woman provided a self-collected and two clinician-collected specimens. The self-collected and one clinician-collected specimen were tested by two HPV DNA tests—*care*HPV™ and Hybrid Capture 2; the other clinician-collected specimen was tested for HPV16/18/45 E6 protein. C*are*HPV™-positive specimens were tested for HPV16/18/45 DNA. HPV DNA-positive women underwent visual inspection with acetic acid (VIA) and then colposcopic evaluation with biopsies. The performance for detection of cervical intraepithelial neoplasia grade 3 or cancer (CIN3+) among HPV DNA-positive women was assessed for different triage strategies: HPV16/18/45 E6 or DNA detection, VIA, colposcopic impression, or higher signal strength (≥10 relative light units/positive control [rlu/pc]). The percent triage positive ranges were 14.8–17.4% for VIA, 17.8–20.9% for an abnormal colposcopic impression; 7.9–10.5% for HPV16/18/45 E6; 23.4–28.4% for HPV16/18/45 DNA; and 48.0–62.6% for higher signal strength (≥10 rlu/pc), depending on the HPV test/specimen combination. The positivity for all triage tests increased with severity of diagnosis. HPV16/18/45 DNA detection was approximately 70% sensitive and had positive predictive values (PPV) of approximately 25% for CIN3+. HPV16/18/45 E6 detection was approximately 50% sensitive with a PPV of nearly 50% for CIN3+. Different triage strategies for HPV DNA-positive women provide important tradeoffs in colposcopy or treatment referral percentages and sensitivity for prevalent CIN3+.

Key words: HPV, triage, cervical cancer, *care*HPV, developing countries, E6

Abbreviations: CI: confidence interval; CICAMS: Cancer Institute and Hospital, Chinese Academy of Medical Sciences; CIN: cervical intraepithelial neoplasia; hc2: hc2 high-risk HPV; HPV: human papillomavirus; IRB: institutional review board; LMIC: low- and middle-income countries; mAb: monoclonal antibodies; OR: odds ratio; Pap: Papanicolaou; START-UP: Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention—Utility and Program Planning Project; VIA: visual inspection with acetic acid

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Cervical cancer incidence and mortality have declined significantly in those places that have effectively implemented Papanicolaou (Pap) test-based screening.[1] Yet cervical cancer remains the second most common female cancer and third most common cause of female cancer-related mortality globally, with an annual incidence of approximately 530,000 and mortality of 275,000, respectively.[2] This seeming contradiction is explained by the fact that cervical cancer incidence and mortality are approximately 10-fold greater in low- and middle-income countries (LMIC), where Pap programs have failed to be established because of the technical and financial barriers to implementation.[1,3]

Because of these limitations, alternative screening strategies have been developed and evaluated, including molecular testing for the necessary cause of cervical cancer, carcinogenic human papillomavirus (HPV). DNA testing for HPV has been shown to be more sensitive[4–9] and more reliable[10–12] than Pap testing. A key attribute of HPV testing related to its high sensitivity is its excellent negative predictive value, providing near complete reassurance following a negative test that the woman does not have cancer or precancer.[13–15] Thus, a negative HPV DNA test does an excellent job of

What's new?

The *care*HPV™ test is a novel technology for primary cervical cancer screening of women from lower-resource settings. However, triage strategies are needed to identify which HPV-positive women are at highest risk of cervical precancer and cancer. Here, multiple viable and affordable strategies to manage HPV-positive women depending on local requirements and resources are identified, based on evaluation of the performance of different triage strategies for developing countries. The different strategies for women who test positive for HPV DNA provide important tradeoffs in colposcopy or treatment referral percentages and sensitivity for cervical intraepithelial neoplasia grade 3 or cancer (CIN3+).

screening by ruling out disease in the primarily healthy population, permitting fewer screens of the general population in lifetime. Affordable tests like *care*HPV™ (*care*HPV™; QIAGEN, Gaithersburg, MD)[16] and "tiered pricing" of higher cost tests will make HPV testing increasingly more available to LMIC. However, the challenge of using HPV testing, or any screening test, is the management of screen-positive women, as most women with a positive screening test (~80% to 90%) will not have concurrent disease (*i.e.*, cervical precancer or cancer). This is an especially perplexing problem in LMIC, where there are limited numbers of clinics, colposcopists, pathologists and clinicians qualified to provide diagnosis and treatment, and services must be prioritized for women at highest risk for harboring precancer or cancer.[17] In China, findings showed a higher prevalence of HR-HPV infection and CIN2+, which suggests that the burden of cervical cancer in China especially in some rural areas is more substantial than was previously reported with a much higher need for comprehensive screening and will result in many more HPV positive women to manage.[18,19] Moreover, in the context of a screen-and-treat program, where treatment is provided without colposcopy or biopsy, it may be desirable to immediately treat only those at a higher risk of cervical precancer and cancer among the screen-positives to minimize overtreatment.

In 2010, we launched a clinical study in 7,500 women living in rural China as part of the Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention—Utility and Program Planning (START-UP) Project. The stated goals of this study were to evaluate new strategies for screening and management of screen positives that might be employed in LMIC. Here, we report on our evaluation of different management or "triage" strategies for HPV DNA-positive women. We explored both visual and molecular methods to distinguish between HPV DNA-positive women at high and low risk of cervical precancer and cancer. Further management of the latter might be deferred until there is evidence of increased risk (*e.g.*, HPV persistence),[15,20,21] thereby increasing the "predictive value" of the intervention and decreasing the use of more invasive procedures and resources in lower-risk women.

Material and Methods

Population

The population for this study was recruited as follows: First, we selected two high-risk communes from each county (Yangcheng, Xinmi and Tonggu) according to the proposed sample size. Second, the number of women aged 25–65 years in each commune was collected from the local residence registry of the police office. Third, we determined the candidate villages for the study considering the size of the village and the transportation conditions. Fourth, all the women aged 25–65 years and living in the chosen villages who had not undergone screening in the last five years were invited to participate in the study if they met the study criteria. The recruitment was stopped when the target sample size was reached. We noted a challenge in the recruitment of the oldest and youngest women as older women were less willing to undergo screening and many of the younger women were transient and could not be located. Thus, our study population was biased toward women who were 35–50 years old.

Women aged 25–65 years were considered eligible if they (*i*) had a cervix, (*ii*) had not been previously diagnosed with cervical cancer, (*iii*) were not pregnant, (*iv*) were physically able to undergo routine cervical cancer screening and (*v*) were able to provide informed consent. We did not exclude women if they had previous cervical cancer screening because we assumed that even if a few women had been screened for cervical cancer, the quality of cytology screening was likely to be very poor. Women were excluded if they were not married and reported never having had sexual intercourse. Local doctors conducted the initial recruitment and eligibility screening. Eligibility was confirmed at the study clinic. Eligible women were then educated about the study and asked to complete the written informed consent in order to participate in the study.

Enrollment visit

Participants were given an education session about cervical cancer prior to the start of the study procedures. First, women were asked to complete a short risk-factor survey administered by study personnel. Then, women were given instructions on how to self-collect a vaginal sample and were provided a private room to self-collect their vaginal specimen. Next, women underwent a routine pelvic exam at which time two cervical specimens were collected, the first into a dry tube for OncoE6™ Cervical Test (Arbor Vita Corporation) testing and the second into dcm buffer (QIAGEN) for HPV DNA testing. Then visual inspection with acetic acid (VIA) was done and results were recorded.

Clinical management

Women who tested positive for any of the six screening tests performed (VIA, HPV E6 and HC2 and *care*HPV^{TM} on clinician-collected and self-collected specimens) were referred to colposcopy and approximately 10% random sample of women who tested negative for all screening tests (screen-negative women) underwent a second VIA and a rigorous colposcopic evaluation that included using a microbiopsy protocol as previously described.[22] As dictated by the IRBs, women who had no visible lesions had their screening result revealed and if there were still no visible lesions, no biopsies were taken.

Laboratory tests

*Care*HPV^{TM} was done as previously described[16,23] at the clinical sites by a laboratory technician who had a general level of training comparable to the local hospital staff and who was trained to run *care*HPV^{TM} by a senior CICAMS technician. A research-use only pooled probe set targeting HPV16, 18 and 45 was developed for the study and ran on the same *care*HPV^{TM} platform with the same protocol on all *care*HPV^{TM}-positive specimens. The HPV DNA 16/18/45 test was run periodically when there were sufficient numbers to nearly or completely fill a batch of 90. A signal strength of 1.0 relative light units per positive control (rlu/pc) or greater was considered positive for both tests.

HC2 was performed per the manufacturer's instruction, except that 50 μL of the dcm specimen was combined with 25 μL kit denaturation reagent rather than combining 1,000 μL of the STM specimen with 500 μL kit denaturation reagent. Because HC2 cannot be set up at local clinical sites, after *care*HPV^{TM} testing, residual dcm specimens were sent to the special lab on site and CICAMS's technician performed the HC2 test.

The Arbor Vita OncoE6^{TM} Cervical Test is an immunochromatographic test using a lateral flow format; E6 oncoproteins of HPV types 16 and/or 18 and/or 45 (when present in the sample solution) are captured by monoclonal antibodies (mAb) immobilized on the porous membrane of the lateral flow strip, thus forming distinct test lines for E6 oncoproteins of the respective HPV types. Detection of captured E6 oncoproteins occurs *via* detector monoclonal antibodies recognizing epitopes on E6 distinct from those used for capture. The detector mAbs are conjugated to alkaline phosphatase, allowing for colorimetric visualization of a present capture mAb–E6 oncoprotein–detector mAb sandwich upon development with alkaline phosphatase substrate. Three test strips constitute one test unit, with each test strip allowing for analysis of one individual clinical specimen, and several units (of three test strips each) can be used in parallel by one operator. The time from sample collection to test results is typically around 2.5 hr. A control line is included on each strip and allows verification of detector reagent activity and proper sample solution migration up the test strip.

OncoE6^{TM} cervical testing was conducted as previously described[24] at the clinical site by local hospital personnel supervised by CICAMS staff. Briefly, a cervical specimen collected using a polyester swab was stored in a tube without buffer until tested. The swab specimen was treated in a two-step process, first with 933 μL of lysis solution and next with 87 μL of conditioning solution, both with 15-minute incubation under gentle agitation. Next, the specimen solution was clarified from insoluble components by centrifugation in a table-top microcentrifuge for 10 minutes at >10,000 rpm. A 200-μL aliquot of the sample solution was then transferred into a vial with lyophilized detector mAb; the test unit was next inserted into the detector mAb vials, and the specimen solutions ran up the test strips by capillary action. After 55 minutes, the test unit was transferred into vials with wash solution, and after a 12-minute washing the test unit was immersed into another set of vials containing developing solution. After 15–25 minutes (depending on the ambient temperature), the test unit was removed from the developing solution vials and placed onto a reading guide, allowing for visual inspection. Appearance of one or more test lines indicated E6 oncoprotein of the corresponding HPV type present in the initial cervical swab specimen.

Pathology

A CICAMS pathologist (Professor Xun Zhang) provided the primary diagnosis of biopsies and surgical specimens, and the worst of the two was used for the final diagnosis in these analyses. All initial biopsy diagnoses of CIN2+ and a random sample of <CIN2 were independently reviewed by an expert US pathologist (MHS) to confirm the results. There was no qualitative difference in the results of this analysis using either set of diagnoses (data not shown).

Analyses

We evaluated five different strategies to triage an HPV-positive test by any of the four combinations of HPV tests (*care*HPV^{TM} or HC2) and specimen collection (clinician or self): (*i*) colposcopic impression of low-grade disease or worse, (*ii*) the second VIA conducted among the screen-positive population referred to colposcopy, (*iii*) using a higher cutpoint of 10.0 rlu/pc for the HPV DNA screening test, (*iv*) the E6 test for HPV16/18/45 and (*v*) the HC2 DNA test for HPV16/18/45 (among *care*HPV^{TM} positives only). We selected a cutpoint for colposcopy based on receiver-operator curve analysis for the detection of CIN2+ shown in Supporting Information Figure 1; using metaplasia or worse added little sensitivity and decreased specificity significantly while using a cutpoint of high-grade or worse was too insensitive. We selected a DNA triage cutpoint of 10.0 rlu/pc based on reports that the signal strength near the 1.0 rlu/pc positive cutpoint were less predictive of CIN2+ and CIN3+, and we wanted to evaluate HPV DNA test performance by reducing the referral by approximately 50%.

We calculated the percent positive of each triage test among any HPV-positive test result overall, by age groups (<30, 30–39, 40–49 and 50 years and older) and by severity

of histologic diagnosis. We calculated sensitivity, specificity, positive and negative predictive values and odds ratio (OR) for CIN2+ and CIN3+ for all triage tests among those women who tested HPV DNA positive. A McNemar chi-square test was used to assess differences in sensitivity and specificity for CIN2+ and CIN3+ for paired-test results.

Results

We recruited 7,543 women aged 25–65 years from the three clinical sites in China; 7,541 (99.9%) were of eligible age—1,935 (25.7%) of whom had at least one positive HPV test and were included in this analysis. The mean age, median age and interquartile age range for this subpopulation (HPV-positive women) were 46 years, 45 years and 39–53 years, respectively. The percentages of test positive for clinician-collected specimens tested by *care*HPV^{TM}, self-collected specimens tested by *care*HPV^{TM}, clinician-collected specimens tested by HC2 and self-collected specimens tested by HC2 were 14.4%, 14.5%, 14.5% and 17.9%, respectively. For HPV DNA detection, the four-way total agreement was 84.4% and the kappa was 0.669.

Among the HPV DNA positives by either specimen or test, the percent positive for each triage test or method was generally similar for all but using a 10.0 rlu/pc or higher cutpoint for the HPV DNA test (Table 1). The range of percent positive for VIA was 14.8–17.4% and for colposcopic impression of low-grade or worse was 17.8–20.9%; HPV16/18/45 E6 detection was 7.9–10.5%, and HPV16/18/45 DNA (among *care*HPV^{TM} positives only) was 23.4–28.4%. By comparison, using a 10 rlu/pc cutpoint for triage, the percent positive was 10% lower on self-collected specimens (48.0% for *care*HPV^{TM} and 52.1% for HC2) than using clinician-collected specimens (58.3% for *care*HPV^{TM} and 62.6% for HC2).

There were consistent patterns of percent positive for the triage tests by age (Table 1): (*i*) both visual methods of triage—colposcopy and VIA—decreased with age, (*ii*) both HPV DNA methods of triage—the 10.0 rlu/pc cutpoint and HPV16/18/45 detection—remained fairly constant with age and (*iii*) E6 detection of HPV16/18/45 increased with increasing age.

The percent positive for the triage tests among HPV DNA-positive women increased with increasing severity of diagnosis (Table 2). The percent positive for the 10 rlu/pc cutpoint was generally higher for any diagnosis than the other triage tests and was relatively constant for diagnoses of CIN1 or more severe. As a consequence, the percent positive (∼50%) for the 10 rlu/pc cutpoint among women with <CIN2 was much greater than colposcopy (∼11%), VIA (∼10%), DNA detection of HPV16/18/45 (∼20%), or E6 detection of HPV16/18/45 (∼5%). It is also noteworthy that there was a large difference in the percent test positive among the 10 rlu/pc cutpoint for CIN2+ and CIN3+ between the clinician- and self-collected specimens. Conversely, there was no appreciable difference in the percent test positive for CIN2+ and CIN3+ for the other triage tests between the different specimens.

The clinical performance of the triage tests among HPV DNA-positive women for CIN2+ is shown in Table 3 and for CIN3+ is shown in Table 4. The 10 rlu/pc cutpoint was generally the most sensitive and least specific of triage tests for both endpoints ($p < 0.0001$) except for the 10 rlu/pc cutpoint for self-collected specimens tested by *care*HPV^{TM}. DNA detection of HPV16/18/45 among *care*HPV^{TM} positives was generally the next most sensitive and the second least specific. E6 detection of HPV16/18/45 was by far the most specific for CIN2+ ($p < 0.0001$ for all) and CIN3+ ($p \leq 0.0001$ for all), and its positive predictive value was greater than 50% for CIN2+ and almost 50% for CIN3+. For visual methods, colposcopy tended to be slightly more sensitive and slightly less specific than VIA, although the differences were not statistically significant. For CIN2+, the sensitivity of colposcopy ranged from 55.3% to 55.8% and the specificity ranged from 84.2% to 86.9%. On the other hand, the sensitivity for VIA ranged between 46.0% and 46.8% and its specificity was between 86.1% and 88.6%. Similar results were observed for the CIN3+ endpoint: the sensitivity for colposcopy was between 63.3% and 63.9% and its specificity was between 83.3% and 86.1%; the sensitivity for VIA ranged from 52.6% to 54.2% and its specificity ranged from 85.3% to 87.9%.

We also calculated the OR and 95% confidence interval (CI) as a summary measure of the association of positive triage tests with the endpoints among HPV DNA-positive women. E6 was the most strongly associated with CIN2+ and CIN3+. For example, E6 detection was strongly associated with CIN3+ among women who tested *care*HPV^{TM}-positive on their clinician-collected specimen (OR: 17.9, 95% CI: 11.1–28.9) and on their self-collected specimen (OR: 28.8, 95% CI: 17.0–48.8). The OR of 10 rlu/pc cutpoint (for clinician-collected specimens only), VIA, colposcopy and DNA for HPV16/18/45 were between 5 and 10 for CIN2+ and between 5 and 15 for CIN3+. At the other extreme, the 10 rlu/pc cutpoint for HC2 on self-collected specimens was only weakly associated with CIN3+ (OR: 2.3, 95% CI: 1.4–3.6) and the 10 rlu/pc cutpoint for *care*HPV^{TM} on self-collected specimens was not associated with CIN3+ (OR: 1.2, 95% CI: 0.8–1.9).

Discussion

We evaluated multiple strategies for managing HPV DNA-positive women (triage) to expand the menu of options for secondary cervical cancer prevention through screening, management of screen positives, diagnosis and early treatment of precancer and early cancer. More sensitive methods of triaging HPV DNA-positive women to detect CIN3+, such as using a higher cutpoint or HPV16/18/45 DNA detection, were generally less specific, *i.e.*, more false positives. Conversely, highly specific methods for CIN3+, such as HPV16/18/45 E6, were less sensitive. In general, the *relative*

Table 1. The percent positive of the triage tests/methods among HPV DNA positives by specimen collection method (clinician or self) and test (*care*HPV™ or Hybrid Capture 2 [HC2]), overall and by age group

	Clinician collection										
	*care*HPV						HC2				
		*care*HPV+						HC2+			
			HPV16/18/45		Colpo				HPV16/18/45		
	n	*care*HPV (≥10)	DNA[1]	E6	impression	VIA (2nd)	n	HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
All	1065	58.3	28.4	10.5	19.9	16.4	1076	62.6	10.5	20.9	17.4
Age group (years)											
<30	46	58.7	30.4	2.2	37.0	30.2	47	53.2	2.1	34.0	27.3
30–39	241	59.8	28.2	7.5	29.9	26.0	240	65.8	7.5	32.5	28.7
40–49	401	58.4	27.2	9.2	20.2	16.3	410	62.4	9.0	21.0	17.2
50+	377	57.3	29.4	14.9	11.1	8.7	379	61.7	15.0	11.9	9.5
	Self collection										
	*care*HPV						HC2				
		*care*HPV+						HC2+			
			HPV16/18/45		Colpo				HPV16/18/45		
	N	*care*HPV (≥10)	DNA[1]	E6	impression	VIA (2nd)	n	HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
All	1074	48.0	23.4	8.7	17.8	14.8	1329	52.1	7.9	18.0	15.1
Age group (years)											
<30	51	47.1	25.5	2.0	27.5	22.5	54	64.8	1.9	29.6	25.0
30–39	232	45.3	23.7	6.0	28.9	25.1	315	47.9	5.4	28.6	24.6
40–49	406	47.8	20.9	8.1	17.5	14.1	525	51.2	7.2	17.0	14.1
50+	385	50.1	25.5	11.7	10.1	8.2	435	54.7	11.3	10.1	8.5

[1] *care*HPV™ test.
Abbreviations: VIA, visual inspection after acetic acid; rlu/pc, relative light units per positive control (signal strength).

Table 2. The percent positive of the triage tests/methods for increasing severity of diagnosis among HPV DNA positives by sampling method (clinician or self) and test (*care*HPV™ or HC2)

*care*HPV+ (clinician)							HC2+ (clinician)					
			HPV16/18/45							HPV16/18/45		
	n	*care*HPV (≥10)	DNA[2]	E6	Colpo Impression	VIA (2nd)		n	HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
Negative[1]	770	47.4	21.3	4.9	11.2	10.0	Negative[1]	771	52.9	4.9	12.2	11.0
CIN1	157	85.4	27.4	9.6	31.2	26.0	CIN1	167	83.8	9.6	32.3	27.5
CIN2	42	88.1	50.0	16.7	38.1	33.3	CIN2	42	90.5	16.7	38.1	33.3
CIN3	83	91.6	74.7	49.4	60.2	50.7	CIN3	83	91.6	49.4	60.2	50.7
Cancer	13	69.2	92.3	84.6	84.6	71.4	Cancer	13	84.6	84.6	84.6	71.4
CIN2+	138	88.4	68.8	42.8	55.8	46.1	CIN2+	138	90.6	42.8	55.8	46.1
CIN3+	96	88.5	77.1	54.2	63.5	52.6	CIN3+	96	90.6	54.2	63.5	52.6
careHPV + (self)							**HC2+ (self)**					
			HPV16/18/45							HPV16/18/45		
	n	*care*HPV (≥10)	DNA[2]	E6	Colpo Impression	VIA (2nd)		n	HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
Negative[1]	811	42.7	16.9	3.1	9.5	8.7	Negative[1]	1033	46.5	3.1	10.4	9.3
CIN1	144	72.9	22.9	9.0	33.3	27.7	CIN1	164	73.8	9.2	36.0	30.8
CIN2	36	58.3	47.2	19.4	36.1	30.3	CIN2	42	69.1	19.1	38.1	33.3
CIN3	72	50.0	73.6	52.8	59.7	50.8	CIN3	79	69.6	50.6	59.5	51.5
Cancer	11	72.7	100.0	90.9	90.9	83.3	Cancer	11	72.7	90.9	90.9	83.3
CIN2+	119	54.6	68.1	46.2	55.5	46.0	CIN2+	132	69.7	43.9	55.3	46.9
CIN3+	83	53.0	77.1	57.8	63.9	53.7	CIN3+	90	70.0	55.6	63.3	54.2

[1]Includes women who did not have biopsies and biopsies that were diagnosed as negative.
[2]*care*HPV™ test.
Abbreviations: CIN, cervical intraepithelial neoplasia; CIN1, CIN grade 1; CIN2, CIN grade 2; CIN3, CIN grade 3, CIN2+, CIN2 or more severe; CIN3+, CIN3 or more severe; VIA, visual inspection with acetic acid; rlu/pc, relative light units per positive control (signal strength).

Table 3. The clinical performance (sensitivity, specificity, PPV and NPV) with 95% confidence intervals of the triage tests/methods for detection of CIN2 or CIN2+ among HPV DNA positives by sampling method (clinician or self) and test (*care*HPV^{TM} or HC2)

	*care*HPV+ (clinician)					HC2+ (clinician)				
		HPV16/18/45						HPV16/18/45		
	*care*HPV (≥10)	DNA	E6	Colpo Impression	VIA (2nd)		HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
Sensitivity	88.4 (81.9, 93.2)	68.8 (60.4, 76.4)	42.8 (34.4, 51.5)	55.8 (47.1, 64.2)	46.1 (36.8, 55.6)	Sensitivity	90.6 (84.4, 94.9)	42.8 (34.4, 51.5)	55.8 (47.1, 64.2)	46.1 (36.8, 55.6)
Specificity	46.2 (42.9, 49.4)	77.7 (74.8, 80.3)	94.3 (92.6, 95.7)	85.4 (83.0, 87.6)	87.4 (85.1, 89.5)	Specificity	41.6 (38.4, 44.8)	94.2 (92.6, 95.6)	84.2 (81.7, 86.5)	86.1 (83.7, 88.3)
PPV	19.6 (16.6, 23.0)	31.5 (26.3, 37.0)	52.7 (43.0, 62.2)	36.3 (29.8, 43.2)	31.5 (24.6, 39.2)	PPV	18.6 (15.7, 21.7)	52.2 (42.6, 61.7)	34.2 (28.0, 40.8)	29.3 (22.8, 63.5)
NPV	96.40 (94.21, 97.93)	94.36 (92.48, 95.89)	91.71 (89.78, 93.38)	92.85 (90.91, 94.49)	92.78 (90.84, 94.42)	NPV	96.77 (94.55, 98.27)	91.80 (89.88, 93.45)	92.83 (90.89, 94.47)	92.77 (90.82, 94.41)
OR	6.5 (3.8, 11)	7.7 (5.2, 11)	12 (8.0, 19)	7.4 (5.1, 11)	5.9 (3.9, 9.0)	OR	6.8 (3.8, 12)	12.2 (7.9, 19)	6.7 (4.6, 9.8)	5.3 (3.5, 8.0)
	*care*HPV+ (self)					HC2+ (self)				
		HPV16/18/45						HPV16/18/45		
	*care*HPV (≥10)	DNA	E6	Colpo Impression	VIA (2nd)		HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
Sensitivity	54.6 (45.2, 63.8)	68.1 (58.9, 76.3)	46.2 (37.0, 55.6)	55.5 (46.1, 64.6)	46.0 (36.0, 56.3)	Sensitivity	69.7 (61.1, 77.4)	43.9 (35.3, 52.8)	55.3 (46.4, 64.0)	46.8 (37.3, 56.6)
Specificity	52.8 (49.6, 56.0)	82.2 (79.6, 84.6)	96.0 (94.6, 97.2)	86.9 (84.6, 89.0)	88.6 (86.4, 90.5)	Specificity	49.8 (46.9, 52.7)	96.1 (94.8, 97.1)	86.1 (84.0, 88.0)	87.9 (85.9, 89.7)
PPV	12.6 (9.86, 15.8)	32.3 (26.5, 38.4)	59.1 (48.5, 69.2)	34.6 (27.8, 41.8)	29.9 (22.8, 37.8)	PPV	13.3 (10.8, 16.0)	55.2 (45.2, 65.0)	30.5 (24.8, 36.8)	26.7 (20.6, 33.5)
NPV	90.32 (87.56, 92.65)	95.38 (93.72, 96.71)	93.48 (91.74, 94.94)	94.00 (92.22, 95.47)	93.93 (92.16, 95.41)	NPV	93.71 (91.53, 95.47)	93.95 (92.47, 95.22)	94.61 (93.10, 95.87)	94.61 (93.10, 95.87)
OR	1.4 (0.92, 2.0)	9.8 (6.5, 15)	21 (13, 34)	8.3 (5.5, 12)	6.6 (4.3, 10)	OR	2.3 (1.6, 3.4)	19 (12, 30)	7.7 (5.3, 11)	6.4 (4.2, 9.6)

Odds ratios with 95% confidence intervals as a measure of association are also shown.
Abbreviations: CIN, cervical intraepithelial neoplasia; CIN2, grade 2; CIN2+, grade 2 or more severe; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; rlu/pc, relative light units per positive control (signal strength); VIA, visual inspection with acetic acid.

Table 4. The clinical performance (sensitivity, specificity, PPV and NPV) with 95% confidence intervals of the triage tests/methods for detection of CIN3 or more severe (CIN3+) among HPV DNA positives by sampling method (clinician or self) and test (*care*HPV™ or HC2)

	*care*HPV+ (clinician)						HC2+ (clinician)				
		HPV16/18/45						HPV16/18/45			
	*care*HPV (≥10)	DNA	E6	Colpo Impression	VIA (2nd)		HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
Sensitivity	88.5 (80.4, 94.1)	77.1 (67.4, 85.0)	54.2 (43.7, 64.4)	63.5 (53.1, 73.1)	52.6 (40.8, 64.2)	Sensitivity	90.6 (82.9, 95.6)	54.2 (43.7, 64.4)	63.5 (53.1, 73.1)	52.6 (40.8, 64.2)
Specificity	44.7 (41.5, 47.9)	76.5 (73.7, 79.1)	93.8 (92.1, 95.2)	84.4 (82.0, 86.6)	86.5 (84.2, 88.6)	Specificity	40.2 (37.1, 43.4)	93.8 (92.1, 95.2)	83.3 (80.8, 85.6)	85.3 (82.9, 87.5)
PPV	13.7 (11.1, 16.6)	24.5 (19.8, 29.8)	46.4 (37.0, 56.1)	28.8 (22.8, 35.4)	23.8 (17.6, 31.0)	PPV	12.9 (10.5, 15.7)	46.0 (36.6, 55.6)	27.1 (21.4, 33.4)	22.1 (16.3, 28.9)
NPV	97.52 (95.61, 98.76)	97.12 (95.67, 98.18)	95.38 (93.85, 96.63)	95.90 (94.34, 97.13)	95.81 (94.24, 97.05)	NPV	97.77 (95.80, 98.97)	95.43 (93.91, 96.66)	95.89 (94.33, 97.12)	95.80 (94.23, 97.04)
OR	6.2 (3.3, 12)	11 (6.7, 18)	18 (11, 29)	9.4 (6.0, 15)	7.1 (4.4, 12)	OR	6.5 (3.3, 13)	18 (11, 29)	8.7 (5.6, 14)	6.5 (4.0, 11)
	*care*HPV+ (self)						HC2+ (self)				
		HPV16/18/45						HPV16/18/45			
	*care*HPV (≥10)	DNA	E6	Colpo Impression	VIA (2nd)		HC2 (≥10)	E6	Colpo Impression	VIA (2nd)
Sensitivity	53.0 (41.7, 64.1)	77.1 (66.6, 85.6)	57.8 (46.5, 68.6)	63.9 (52.6, 74.1)	53.7 (41.1, 66.0)	Sensitivity	70.0 (59.4, 79.2)	55.6 (44.7, 66.0)	63.3 (52.5, 73.2)	54.2 (42.0, 66.0)
Specificity	52.4 (49.2, 55.5)	81.1 (78.6, 83.5)	95.5 (94.0, 96.7)	86.1 (83.8, 88.2)	87.9 (85.7, 89.9)	Specificity	49.2 (46.3, 52.0)	95.6 (94.3, 96.6)	85.3 (83.2, 87.2)	87.2 (85.2, 89.0)
PPV	8.53 (6.26, 11.3)	25.5 (20.2, 31.4)	51.6 (41.0, 62.1)	27.7 (21.5, 34.7)	23.4 (16.9, 30.9)	PPV	9.09 (7.06, 11.5)	47.6 (37.8, 57.6)	23.8 (18.6, 29.8)	20.0 (14.6, 26.3)
NPV	93.01 (90.57, 94.98)	97.69 (96.42, 98.60)	96.43 (95.07, 97.50)	96.60 (95.18, 97.70)	96.52 (95.09, 97.62)	NPV	95.75 (93.88, 97.18)	96.73 (95.58, 97.66)	96.97 (95.77, 97.91)	96.99 (95.79, 97.92)
OR	1.2 (0.79, 1.9)	15 (8.5, 25)	29 (17, 49)	11 (6.8, 18)	8.5 (5.1, 14)	OR	2.3 (1.4, 3.6)	27 (16, 44)	10 (6.4, 16)	8.1 (4.9, 13)

Odds ratios (OR) with 95% confidence intervals as a measure of association are also shown. Abbreviations: CIN, cervical intraepithelial neoplasia; CIN2, grade 2; CIN3, grade 3; CIN2+, grade 2 or more severe; CIN3+, grade 3 or more severe; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; rlu/pc, relative light units per positive control (signal strength); VIA, visual inspection with acetic acid.

performance of the different management strategies did not depend on the method of screening to identify HPV-positive women.

Appropriate screening strategies for LMICs differ from the criteria for strategies appropriate for a high-income country.[25] Screening programs, including the management strategies, will need to be tailored to meet local needs in terms of financial and human resources, infrastructure and capacities, societal norms and patient acceptability and level of cancer risk reduction desired. For instance, the Chinese government launched a cervical cancer prevention program targeting 10 million rural women using a Pap smear or VIA during the year of 2009–2011.[26] However, China lacks a sufficient number of cytopathologists or trained health care workers to screen an estimated 500 million women in rural areas by Pap smear or VIA. Objective, efficient and high reproducible screening tests are more desirable in China. The affordable HPV DNA test (*care*HPV^{TM}) has been developed for developing countries.[16] Testing with *care*HPV^{TM} at five-year intervals was reported to be the optimal cervical cancer screening strategy for China and has the best cost-effectiveness performance and the highest benefit-cost ratio with moderate life outcomes in a modeling study.[27] The decision to triage and by what method will depend greatly on the weighting of the benefits (*e.g.*, cancer prevention), the harms (*e.g.*, false positive results leading to unnecessary colposcopy, biopsy and possibly treatment) and programmatic issues (*e.g.*, losses to follow-up and availability of health services) in the local context.

HPV DNA testing is uniquely suited as a screening test because of its ability to "rule out" disease (*i.e.*, women who test negative for the necessary causal factor, HPV, are on average many years away from developing invasive cervical cancer).[28] One of the challenges to widespread implementation of HPV DNA testing is its lower specificity. Some countries may have insufficient capacity to act (*e.g.*, colposcopy or immediate treatment) on a HPV DNA-positive result. In such places, strategies to prioritize women into higher-risk groups in need of immediate intervention and lower-risk groups who might be deferred for a period of time (*e.g.*, 6–24 months) and then re-evaluated would be valuable.

Here we presented a variety of methods/tests that might be employed to make the distinction between higher- and lower-risk HPV DNA-positive women. Each method has distinct advantage(s) in terms of its performance and/or applicability. Simply using a higher cutpoint of 10 rlu/pc as the triage, with $\geq$10 rlu/pc for immediate intervention and 1.0–9.9 rlu/pc deferred to follow-up, could be seen perhaps as the easiest to implement because it requires no additional tests, if the rlu/pc value was made available. Previous findings of primary screening strategies showed that the performance of HPV DNA testing with an increased cutoff-point of 10 rlu/pc may be ideal for a population such as China that could utilize a single test primary screening strategy to allow for infrequent screening and minimal infrastructure requirements.[25,29] In this study using a cutpoint of 10 rlu/pc as the triage was the most sensitive and least specific triage for CIN2+ and CIN3+ when using a clinician-collected specimen but was much less effective when using a self-collected specimen, such that it was no better than chance when tested with *care*HPV^{TM}.

Detection of HPV16/18/45 using a research-use only assay on the *care*HPV^{TM} platform was very sensitive, identifying nearly 70% of all CIN2+ and nearly 80% of all CIN3+, and had positive predictive value (PPV) around 25%, as predicted by the etiologic fraction of cervical cancer and precancer caused by these three HPV genotypes.[30,31] Our findings are consistent with data reported for a recently US Food and Drug Administration-approved HPV DNA test that includes HPV16 and HPV18 detection and previous reports from epidemiologic studies of the clinical utility of HPV16 or HPV16 and 18 detection.[14,15,32] Newly developed US screening guidelines have reaffirmed the use of HPV16 or HPV16 and HPV18 detection for management of HPV-positive, Pap-negative women.[33] Since HPV16/18/45 DNA testing was done reflexively from a *care*HPV^{TM}-positive specimen, there was no additional specimen or visit required, making it relatively simple to implement. One caveat is that in order to maximize its cost-effectiveness, HPV16/18/45 DNA testing on this platform would need to be done in batches, which could take several days to accumulate enough specimens and may preclude same-day "screen-and-intervene" programs in clinics with smaller numbers of women or a low HPV prevalence.

Visual methods of triage, such as colposcopy and VIA, had lower sensitivity and better specificity for CIN2+ and CIN3+ than DNA methods of triage. Colposcopy was more sensitive but less specific than VIA. These methods could be done on the same day or with a subsequent visit and followed by the appropriate intervention (diagnosis and/or treatment). Not surprisingly, visual methods have been proposed as the triage method for HPV DNA testing in low-resource settings, especially since VIA is already being used in many countries. A review of the criteria used for VIA or colposcopy could be required when working with women known to be infected with carcinogenic HPV types.

We noted that the VIA performance was less sensitive but more specific in this setting than reported in recent meta-analyses/systematic reviews.[34,35] We also observed that colposcopy missed approximately 35% of disease (*i.e.*, 35% of disease was found by random biopsies after negative colposcopic assessment). Given the possible bias of using colposcopy to assess VIA performance,[36] we suggest that in order to accurately assess the true performance of VIA, random biopsies are required to identify lesions not visually apparent.

The detection of HPV16/18/45 E6 had sensitivity comparable to VIA but had the best specificity of any triage test or method and predicted that one out of every two HPV16/18/45 E6 positives had clinically important disease (CIN3+). Thus, in the programmatic context in which referral to colposcopy or overtreatment in the context of a screen-and-treat program needed to be minimized, detection of HPV16/18/45

E6 by OncoE6^{TM} Cervical Test might be one option to consider. One caveat is that OncoE6^{TM} Cervical Test only targets HPV16, 18 or 45; targeting additional types is expected to increase sensitivity without significant impact on specificity, since E6 overexpression is expected to be a characteristic of precancer of any type rather than infection. Another limitation is that a second dedicated sample was needed for testing, so either co-collection of a clinician specimen for OncoE6^{TM} Cervical Testing or a second visit of the HPV DNA-positive women would be required. Adapting the OncoE6^{TM} Cervical Test for use with specimens stored in buffers/transport medium employed for HPV DNA testing would increase its usability as a triage method.

As noted above, the sensitivity of the 10 rlu/pc cutoff for triage dropped significantly with the use of the self-collected specimen compared to the clinician-collected specimen. The consequence of this is that the 10 rlu/pc cutoff is a significantly more sensitive triage method using the clinician-collected specimen ($p < 0.0001$ for CIN3+) but less sensitive using the self-collected specimen ($p = 0.0003$ for CIN3+) compared to HPV16/18/45 DNA detection. A comparison of signal strength (rlu/pc) and HPV16/18/45 detection (Supporting Information Table 1) shows that low signal strength (1−<10 rlu/pc), HPV16/18/45 DNA-positive results from the clinician-collected specimens identified fewer cases of CIN2+ and CIN3+ than high signal strength (≥10 rlu/pc), HPV16/18/45 DNA-negative results but the converse was true for self-collected specimens.

We acknowledge the following limitations in our study. First, we did not conduct cytology, which is one of the standard methods being considered for triage of HPV positives in higher-resource countries. Collection of a third cervical specimen (fourth specimen overall) would have been necessary to include cytology in the study and we could not be sure of the quality of the last specimen. The strengths and weaknesses of cytologic methods have been well documented and described, and therefore we felt justified in not studying it again. Second, everyone who was HPV DNA positive went immediately to colposcopy, and therefore we did not evaluate the triage tests/methods as they would actually be used in clinical practice, with a negative triage deferred from evaluation until a follow-up visit (*e.g.*, one year). The consequence of this design is that some CIN2 that might have otherwise regressed[37,38] in the triage-negative group was detected and treated immediately and a small number of CIN3 could have developed into invasive cancer during the time interval.

Our findings show that increasing the evidence and knowledge about these programmatic choices will enable countries, specifically LMICs, to make an informed decision about what strategies might work best for screening their women. The next step forward is large, realistic demonstrations to show how assembling these different components into a cogent program can perform in the real world.

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