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Research Article

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An Evaluation of Novel, Lower-Cost Molecular Screening Tests for Human Papillomavirus in Rural China

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Abstract

New, lower-cost tests that target high-risk human papillomavirus (HR-HPV) have been developed for cervical cancer screening in lower-resource settings but large, population-based screening studies are lacking. Women ages 25 to 65 years and living in rural China ($n = 7{,}543$) self-collected a cervicovaginal specimen, had 2 cervical specimens collected by a clinician, and underwent visual inspection after acetic acid (VIA). The self- and one clinician-collected specimens underwent HR-HPV DNA testing by *care*HPV (QIAGEN) and Hybrid Capture 2 (HC2; QIAGEN) and the other clinician-collected specimen was tested for HPV16, 18, and 45 E6 using OncoE6 (Arbor Vita Corporation). Women who screened positive for any test and a random sample of those negative on all tests underwent colposcopic evaluation. The percent test positive was 1.8% for HPV E6 oncoprotein, between 14% and 18% for HR-HPV DNA testing, and 7.3% for VIA. The sensitivity for cervical intraepithelial neoplasia grade 3 or more severe (CIN3$^+$; $n = 99$) was 53.5% for OncoE6, 97.0% for both *care*HPV and HC2 testing of the clinician-collected specimen, 83.8% for *care*HPV testing and 90.9% for HC2 testing of the self-collected specimen, and 50.5% for VIA. OncoE6 had the greatest positive predictive value (PPV), at 40.8% for CIN3$^+$, compared with the other tests, which had a PPV of less than 10%. OncoE6 tested 70.3% positive for HPV16, 18, or 45-positive CIN3$^+$ and tested negative for all HPV16-, 18-, or 45-negative CIN3$^+$ ($P < 0.0001$). HPV E6 oncoprotein detection is useful for identifying women who have cervical precancer and cancer. *Cancer Prev Res; 6(9); 938–48. ©2013 AACR.*

Introduction

The unequal burden of cervical cancer in resource-limited populations stems primarily from well-known limitations of Pap testing (1). Thus, the development and validation of novel, low-cost, and robust screening strategies are much needed if the unequal burden of cervical cancer worldwide is to be addressed.

Identification of high-risk human papillomavirus (HR-HPV) as the obligate cause of cervical cancer has led to the development of molecular assays that target the virus. DNA testing for HR-HPV provides improved, more reliable identification of women with cervical precancer, and cancer than Pap testing (2–7). The increased sensitivity of HR-HPV testing over Pap testing translates into 2 important health care benefits: (i) earlier detection of precancerous lesions that, if treated, results in a reduced incidence of cervical cancer within 4 to 5 years (5, 6) and reduced death within 8 years (8); and (ii) greater reassurance against cancer (lower cancer risk) following a negative result for many years (9, 10), which permits screening at an extended interval.

Limitations of DNA testing for HR-HPV include its complexity and cost and its inability to differentiate between low-risk and high-risk HPV infections. To address the first limitation and increase access to screening, lower-cost tests have been developed and are undergoing validation studies. The first of these is *care*HPV (QIAGEN), a signal amplification DNA test for a pool of 14 HR-HPV genotypes. Preliminary studies of *care*HPV have shown sensitivity and specificity that approach that of Hybrid Capture 2 (HC2; QIAGEN; ref. 11), a U.S. Food and Drug Administration-approved test, and can be run by secondary school graduates without laboratory experience, using a training of the trainer model (12).

A second lower-cost test that targets HPV E6 oncoproteins (OncoE6, Arbor Vita Corporation) has been developed. HPV E6, along with E7, is the main mediator of oncogenic potential. Overexpression of E6 is a characteristic of the precancerous phenotype (13). The prototype test is a lateral flow immunoassay (14) that detects the E6 oncoprotein

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from HPV16, 18, and 45, the 3 carcinogenic HPV genotypes that cause approximately 75% of cervical cancer (15).

Larger-scale evaluations of both lower-cost tests and strategies on how to use them are currently lacking. To address this gap, we conducted a clinical trial in 7,500 women living in rural China. The goal of this first report from this cohort is to characterize the screening performance (sensitivity, specificity, and predictive values) of multiple screening options using these lower-cost tests, including the first trial of the OncoE6 test.

Materials and Methods

Population

We targeted a sample size of 7,500 to diagnose an expected number of 200 cervical intraepithelial neoplasia grade 2 or higher ($CIN2^+$), which would have allowed us to describe the expected E6 sensitivity for $CIN2^+$ of 40% to 60% with a precision of at least $\pm$7%. A retrospective sample size calculation shows that for 144 cases, the precision for the sensitivity of E6 testing was approximately $\pm$8%. The population for this study was recruited as follows: First, we selected 2 communes where most of the women have never been screened from each county (Yangcheng, Xinmi, and Tonggu) according to the proposed sample size. Second, the number of women aged 25 to 65 years in each village in the 2 communes was collected from the local residence registry of the police office. Third, we determined the candidate villages for the study considering the size of village and the transportation situation. Fourth, all the women aged 25 to 65 living in the chosen village were invited to participate in the study if they met the criteria. The recruitment was stopped when the target sample size was reached. In total, 11,359 eligible women were identified in the 3 counties and 7,543 recruited into the study. Recruitment for the entire cohort took place from October 2010 through June 2011.

Women ages 25 to 65 years old were considered eligible if they (i) had not been previously diagnosed with cervical cancer; (ii) had a cervix; (iii) were not pregnant; (iv) were physically able to undergo routine cervical cancer screening; and (v) were able to provide informed consent. 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. All eligible women were then asked to complete the written, informed consent to participate in the study. Women were provided with an overview of the study and education on cervical cancer before signing consent.

The PATH, Cancer Institute and Hospital, Chinese Academy of Medical Sciences (CICAMS), and US National Cancer Institute institutional review boards (IRB) approved the study.

Enrollment visit

Participants were given an education session about cervical cancer before the start of the study procedures. First, women were asked to complete a short risk factor questionnaire administered by study personnel. Then, women were given instructions on how to self-collect a vaginal specimen; the procedure was completed in private room. Next, women underwent a routine pelvic exam by female clinicians, at which time 2 cervical specimens were collected, the first into a dry tube for OncoE6 testing and the second into *care*HPV collection medium (CCM; QIAGEN) for HR-HPV DNA testing; finally visual inspection after 5% acetic acid (VIA) was done and results recorded.

Clinical management

Women who tested positive for any of the 6 screening tests conducted (VIA, HPV E6, and HC2 and *care*HPV on clinician-collected and self-collected specimens) were referred to colposcopy, and approximately 10% random sample of the women who tested negative for all screening tests (screen-negative women) underwent a rigorous colposcopic evaluation that included using a biopsy protocol as previously described (16). As dictated by the IRBs, women who had no visible lesions had their screening result revealed and if there were no visible lesions, no biopsies were taken.

Laboratory tests

*Care*HPV was done as previously described (11, 12) at the clinical sites by a laboratory technician who had a general level of training comparable with the local hospital staff and had been trained to run *care*HPV by a senior CICAMS technician. A research-use only pooled probe set targeting HPV16, 18, and 45 was developed for the study and run on the same *care*HPV platform with the same protocol on all *care*HPV-positive specimens. Signal strength of 1.0 or greater was considered positive for both tests.

HC2 was conducted on the *care*HPV specimen collected in CCM using a modified manufacturer's instruction protocol by the CICAMS technicians from Beijing. Rather than denaturing the specimens, as per the manufacturer's instruction, 50 μL of the CCM specimen was combined with 25 μL kit denaturation reagent and denatured in a hybridization plate in a microplate heater.

The Onco E6 cervical test is an immunochromatographic test using lateral flow format conducted by local hospital personnel supervised by CICAMS staff using a protocol as previously described (14). Three test strips constitute one test unit, with each test strip allowing for analysis of one individual clinical specimen and several units (of 3 test strips each) can be used in parallel by one operator. A control line is included on each strip, which allows for verification of detector reagent activity and proper sample solution migration up the test strip. The time from sample collection to test results is typically approximately 2.5 hours.

Briefly, a cervical specimen collected using a polyester swab was stored in a tube without buffer until tested. The specimen was prepped sequentially by treating the swab with a lysis solution (15 minutes), a condition solution (15 seconds), and then clarifying the specimen solution using a table-top microcentrifuge (10 minutes at >10,000 rpm). A

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0.2 mL aliquot of the clarified specimen solution is then transferred into a vial with lyophilized detector monoclonal antibody alkaline–phosphatase conjugate. The test strips with immobilized capture monoclonal antibodies are inserted into specimen-conjugate mixture and the solution is permitted to migrate up the strip by capillary action. After 55 minutes, the tests are washed for 12 minutes and then immersed into the developing solution containing the alkaline–phosphatase substrate (Nitroblue Tetrazolium). After 15 to 25 minutes (depending on the ambient temperature), the test unit is removed from the developing solution vials and placed on a reading guide, allowing for visual inspection. Appearance of one or more test lines indicates E6 oncoprotein of the corresponding HPV type present in the initial cervical swab specimen.

Pathology

The primary histopathologic diagnosis was provided by 2 CICAMS (Beijing, PR China) pathologists after reaching an agreement and the worst of the biopsies or surgical specimen was used for the final diagnosis in these analyses. All initial biopsy diagnoses of $CIN2^+$ were independently reviewed by an expert U.S. pathologist (M.H. Stoler) to confirm the results. The results of the 2 independent reads are shown in Supplementary Table S1. There was no qualitative difference in the results of this analysis using either set of diagnoses (data not shown). Additional sections of all initial biopsy diagnoses of $CIN2^+$ were cut and tested for $p16^{INK4a}$ by immunohistochemistry as previously described (17).

Genotyping

The biopsied tissues diagnosed as $CIN2^+$ underwent HPV genotyping for HPV genotype-specific attribution by PCR using SPF_{10} primers, which amplify a 65 bp region in L1, as previously described (17, 18). After PCR, 10 μL of the amplimers that were positive for HPV DNA in the DNA immunoassay were used in the reverse hybridization line probe assay ($LiPA_{25}$; version 1, Laboratory Biomedical Products). $LiPA_{25}$ can be used to detect 25 high-risk and low-risk HPV types (15, 19), including HPV16, 18, and 45. HPV-positive specimens that tested negative by $LiPA_{25}$ genotyping were designated as HPV positive but no HPV genotype detected and classified as positive for noncarcinogenic HPV in the analyses. Biopsy specimens that were HPV DNA negative were retested on 1:10 dilution (1:100 dilution overall). If both of the DEIA tests were negative, another aliquot was spiked with plasmid containing HPV16 L1 and the probe of HPV16, which was tested using a HPV16 type-specific test. The samples were considered HPV negative if HPV16 type-specific tests on the plasmid-spiked sample were positive. Otherwise, the tests were considered as failures due to PCR inhibition.

Statistical methods

Standard contingency table methods with Pearson χ^2 tests were used to assess differences in risk factors and sociodemographics and percent test positive for the 3 study locations. Where noted, a test of trend (20) in percent test positive was used.

We calculated sensitivity, specificity, and positive and negative predictive values for all screening tests. Because of the restrictions in taking biopsies from women who were negative on all 6 screening tests, of the 485 screen-negative women who went to colposcopy, only 22 (4.5%) were biopsied and none had $CIN2^+$ [0.0%; 95% confidence interval (CI), 0.0–15.4]. We therefore did not make any adjustments for verification biases. A McNemar χ^2 test was used to assess differences in sensitivity and specificity for $CIN2^+$ and $CIN3^+$ for paired test results.

Results

Figure 1 shows the CONSORT diagram of the participants in the study. Of the 7,543 recruited into the study, 7,539 (99.9%) were age eligible and had valid OncoE6 cervical test results. A total of 2,290 women tested positive by at least one of the screening tests (30.4%) and were

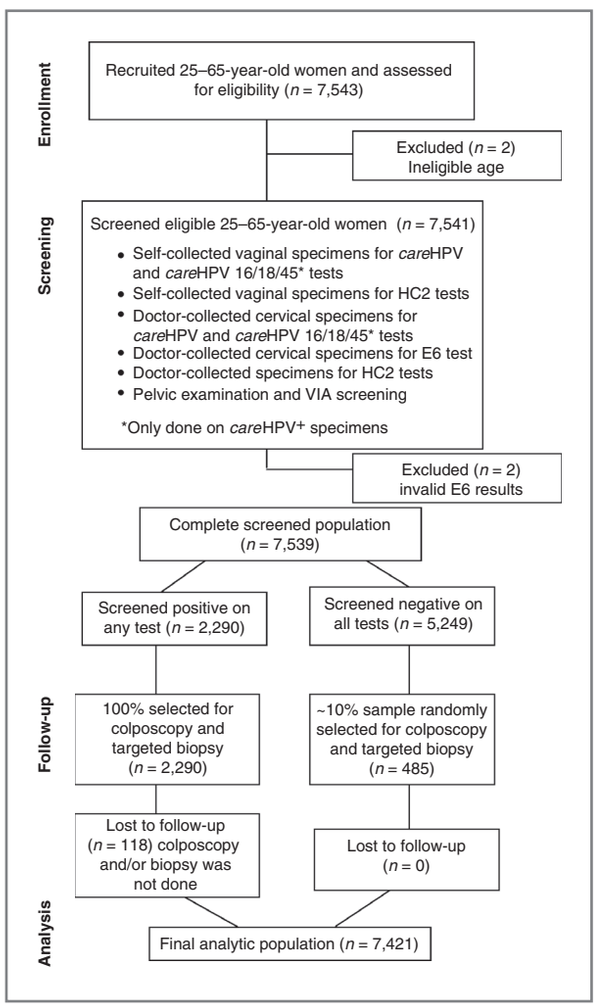

Figure 1. CONSORT diagram for the study.

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referred to colposcopy; 118 (5.2% of 2,290) did not undergo colposcopy and/or have biopsies. The remaining 5,249 (69.6%) tested negative by all tests (screen-negative). A random sample of 9.2% of the screen-negative women ($n = 485$) was referred to colposcopy and all complied, of whom 22 had visible lesions that were biopsied but no CIN2$^+$. The final analytic cohort was of 7,421 women (98.4%).

The sociodemographics and risk factors overall and for each site individually are shown in Table 1. Most women were married (97.1%), of the Han ethnic group (99.9%), never used oral contraceptives (93.8%), and never smoked (99.6%). Most of the characteristics differed statistically but many were not qualitatively different between sites.

Table 2 shows the percent test positive and prevalence of CIN2$^+$ and CIN3$^+$ by clinical site. Overall, the percent test positive was 1.8% for HPV E6, 14.4% and 14.5% for clinician- and self-collected specimens tested by *care*HPV, 14.5% and 17.9% for clinician- and self-collected specimens tested by HC2, and 7.3% for VIA. The percent test positive was the highest for all 6 screening tests in Tonggu, especially for VIA, which was approximately twice as likely to be positive as at the other sites (11.9% vs. 6.2% for Yangcheng and 5.4% for Xinmi). Also, the prevalence of CIN2$^+$ (3.0%) and CIN3$^+$ (1.9%) in Tonggu was almost twice the prevalence seen in the other clinical sites.

The percent test positive increased significantly with increasing severity of diagnosis for all 6 screening tests (Table 3), but the patterns were very distinct for each category of marker/biomarker. The 4 measures of HR-HPV DNA, 2 tests and the 2 collection methods, had a percent test positive of 10% to 15% in women with negative or no histology, 75% to 90% in women with a CIN1 diagnosis, and 80% to 100% for diagnoses of CIN2, CIN3, and cancer. Notably, there was a much higher percent test positive for HC2 testing of self-collected specimens than any other combination of HPV test and sample ($P < 0.001$ for all).

The percent E6 positive increased steadily with increasing severity of diagnosis: 0.8% for negative or no histology, 8.5% for CIN1, 17.8% for CIN2, 48.8% for CIN3, and 84.6% for cervical cancer. In comparison, the percent VIA positive was 7 times more than E6 (5.9% vs. 0.8%, $P < 0.0001$) among women with negative or no histology and almost 4 times more than E6 (30.9% vs. 8.5%, $P < 0.0001$) among women with CIN1.

In Table 4, we stratified the E6 results in CIN2, CIN3, cervical cancer, CIN2$^+$, and CIN3$^+$ by the HPV genotype detected in the lesions tissue and compared the E6 results with DNA detection of HPV16/18/45 conducted among *care*HPV-positive women (clinician-collected specimen). None (95% CI, 0.0–7.5) of 47 HPV16/18/45-negative CIN2$^+$ women tested positive by HPV E6, whereas 5 (15.7%; 95% CI, 8.2–28.0) tested positive for HPV16/18/45 DNA at genotyping. The sensitivity of OncoE6 increased when restricted to CIN2$^+$ positive to genotypes 16/18/45 compared with all CIN2$^+$ (64.5% vs. 42.4%, respectively) and CIN3$^+$ positive to genotypes 16/18/45 compared with all CIN3$^+$ (70.3% vs. 53.5%, respectively). The percent HPV E6 was nonsignificantly greater for p16^{INK4a}-positive versus p16^{INK4a}-negative HPV16/18/45-positive CIN2$^+$ (67.1% vs. 37.5%, $P = 0.16$) and CIN3$^+$ (73.1% vs. 42.9%, $P = 0.25$).

The clinical performance for CIN2$^+$ and CIN3$^+$ of the 6 screening tests is presented in Table 5 and site-by-site performance is shown in Supplementary Table S2. Clinician-collected specimens tested for HR-HPV DNA by HC2 and *care*HPV were the most sensitive for CIN2$^+$ (95.8% for both) and CIN3$^+$ (97.0% for both), and the sensitivity for CIN2$^+$ and CIN3$^+$ was more with the clinician-collected specimens than with self-collected specimens for HC2 (95.8% vs. 91.7% for CIN2$^+$, $P = 0.2$; 97.0% vs. 90.9% for CIN3$^+$, $P = 0.06$) and *care*HPV (95.8% vs. 82.6% for CIN2$^+$, $P < 0.0001$; 97.0% vs. 83.8% for CIN3$^+$, $P = 0.001$). The HR-HPV DNA testing was the least specific for CIN2$^+$ and CIN3$^+$, with specificities in the mid-80% range.

The OncoE6 was 42.4% sensitive for CIN2$^+$ and 53.5% sensitive for CIN3$^+$. However, the OncoE6 was very specific, at 99% for CIN2$^+$ and CIN3$^+$, resulting in a very high PPV for CIN2$^+$ (46.9%) and for CIN3$^+$ (40.8%) compared with HR-HPV DNA detection methods (i.e., 10%–13% PPV for CIN2$^+$ and 7%–9% for CIN3$^+$). In comparison, VIA was equally sensitive for CIN2$^+$ and CIN3$^+$ ($P = 0.5$ for CIN2$^+$ and $P = 0.8$ for CIN3$^+$) but much less specific for CIN2$^+$ and CIN3$^+$ ($P < 0.0001$ for specificity for both endpoints) than the OncoE6 and its PPVs for CIN2$^+$ and CIN3$^+$ were comparable with HR-HPV DNA detection in this study.

Finally, we examined some of the effects of age on our performance metrics (Fig. 2). As previously reported, the prevalence (percent-positive) of HR-HPV DNA by both tests on both specimens (clinician- and self-collected) remained high ($\geq$12%) at all ages, as previously reported for China (18) and the highest percent positive was in women aged 50 years and older (Fig. 2A). The sensitivity for CIN2$^+$ and CIN3$^+$ (Fig. 2B and C) remained high for all HR-HPV DNA methods at all ages, but there was a notable, albeit slight, nonsignificant decline in performance with increasing age for both HR-HPV DNA tests using both specimens. In contrast, the percent E6 positive ($P_{trend} = 0.003$) and E6 sensitivity for CIN2$^+$ ($P_{trend} = 0.02$) and for CIN3$^+$ ($P_{trend} = 0.3$) increased with increasing age, whereas the percent VIA positive ($P_{trend} < 0.0001$), VIA sensitivity for CIN2$^+$ ($P_{trend} < 0.0001$), and sensitivity for CIN3$^+$ ($P_{trend} < 0.0001$) decreased sharply with age. The PPVs for all screening tests were relatively independent of age (Fig. 2D).

Discussion

We observed in our study that (i) HPV E6 oncoprotein detection by OncoE6 was very specific for the presence of cervical precancer and cancer, especially CIN3$^+$ caused by the targeted HPV genotypes and as result, had a remarkable PPV in a screening population; (ii) HR-HPV DNA testing of the clinician-collected specimen by both HC2 and *care*HPV

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Table 1. Sociodemographics and risk factors for all 7,421 women included in the study and for women enrolled at the individual clinical sites

	All $n = 7,421$		Yangcheng $n = 3,180$		Xinmi $n = 2,463$		Tonggu $n = 1,778$		
	n	Col %	*n*	Col %	*n*	Col %	*n*	Col %	*P*
Age, y									
25–29	327	4.4	176	5.5	13	0.5	138	7.8	<0.001
30–39	1,954	26.3	932	29.3	439	17.8	583	32.8	
40–49	2,994	40.4	1,095	34.5	1,221	49.6	678	38.1	
50+	2,146	28.9	977	30.7	790	32.1	379	21.3	
Ethnicity									
Han	7,416	99.9	3,177	99.9	2,463	100.0	1,776	99.8	0.242
Hui	4	0.1	3	0.1	0	0.0	1	0.1	
Other	1	0.0	0	0.0	0	0.0	1	0.1	
Education									
No formal schooling	797	10.7	185	5.8	452	18.4	160	9.0	<0.001
Primary school only	2,679	36.1	1,103	34.7	720	29.2	856	48.1	
Junior high school	3,118	42.0	1,476	46.4	1,044	42.4	598	33.6	
Senior high school	696	9.4	345	10.9	222	9.0	129	7.3	
College or University	131	1.8	71	2.2	25	1.0	35	2.0	
Occupation									
Worker	383	5.2	69	2.2	133	5.4	181	10.2	<0.001
Farmer	5,591	75.3	2,836	89.2	2,100	85.3	655	36.8	
Professional	320	4.3	178	5.6	79	3.2	63	3.6	
Service provider	132	1.8	44	1.4	42	1.7	46	2.6	
Office worker	46	0.6	16	0.5	10	0.4	20	1.1	
Other	949	12.8	37	1.2	99	4.0	813	45.7	
Number of house occupants									
0 to 3	2,183	29.4	1,439	45.3	376	15.3	368	20.7	<0.001
4	2,708	36.5	1,206	37.9	888	36.0	614	34.5	
5	1,182	15.9	306	9.6	512	20.8	364	20.5	
>5	1,348	18.2	229	7.2	687	27.9	432	24.3	
Income									
0 to 3,000	2,626	35.4	1,463	46.0	270	10.9	893	50.2	<0.001
3,001 to 5,000	2,258	30.4	919	28.9	788	32.0	551	31.0	
5,001 to 6,000	704	9.5	130	4.1	541	22.0	33	1.9	
6,001+	1,799	24.2	668	21.0	830	33.7	301	16.9	
Missing	34	0.5	0	0.0	34	1.4	0	0.0	
Smoking									
Never	7,392	99.6	3,174	99.8	2,460	99.9	1,758	98.9	<0.001
Current	25	0.3	3	0.1	3	0.1	19	1.1	
Past	4	0.1	3	0.1	0	0.0	1	0.0	
Marital status									
Single	3	0.0	3	0.1	0	0.0	0	0.0	
Married	7,205	97.1	3,084	97.0	2,402	97.5	1,719	96.7	<0.001
Cohabitating	7	0.1	0	0.0	5	0.2	2	0.1	
Divorced	23	0.3	8	0.3	5	0.2	10	0.6	
Separated	6	0.1	1	0.0	1	0.1	4	0.2	
Widowed	177	2.4	84	2.6	50	2.0	43	2.4	
Number of pregnancies									
0 to 2	2,769	37.3	1,566	49.2	625	25.4	578	32.5	<0.001
3	2,255	30.4	948	29.8	783	31.8	524	29.5	

(Continued on the following page)

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Table 1. Sociodemographics and risk factors for all 7,421 women included in the study and for women enrolled at the individual clinical sites (Cont'd)

	All $n = 7,421$		Yangcheng $n = 3,180$		Xinmi $n = 2,463$		Tonggu $n = 1,778$		
	n	Col %	*n*	Col %	*n*	Col %	*n*	Col %	*P*
4	1,391	18.7	470	14.8	582	23.6	339	19.0	
5+	1,006	13.6	196	6.2	473	19.2	337	19.0	
Oral contraceptive use									
Never	6,957	93.8	2,927	92.0	2,388	97.0	1,642	92.4	<0.001
Former	187	2.5	156	4.9	7	0.3	24	1.3	
Current	37	0.5	16	0.5	2	0.1	19	1.1	
Missing	240	3.2	81	2.6	66	2.6	93	5.2	
Number of sexual partners (lifetime)									
1	6,476	87.3	2,640	83.0	2,298	93.3	1,538	86.5	<0.001
2+	945	12.7	540	17.0	165	6.7	240	13.5	
Number of sexual partners (last 6 months)									
0	770	10.4	315	9.9	217	8.8	238	13.4	<0.001
1	6,569	88.5	2,809	88.3	2,240	91.0	1,520	85.5	
2+	82	1.1	56	1.8	6	0.2	20	1.1	

NOTE: We noted challenges in 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 aged 35 to 50 years. Differences in the distributions between sites were tested for statistical significance using the Pearson χ^2 test.

was very sensitive for CIN2$^+$ and CIN3$^+$ but not specific due to the high prevalence of HPV infection at all ages, as previously reported (21); and (iii) the sensitivity for CIN2$^+$ and CIN3$^+$ of HR-HPV DNA testing of self-collected specimens was very good but less than using clinician-collected specimens. We observed that the decrement in sensitivity was more for *care*HPV testing than for HC2; however, self-sampling does not reduce specificity for *care*-HPV and only slightly for HC2.

The specificity of the OncoE6 for cervical precancer and cancer raises the possibility of its application to screening high HPV-prevalence, high-risk populations, such as for cervical (21–25) and anal cancer (26) in HIV-infected women and anal cancer in HIV-infected (27–29) and -uninfected men (30) who have sex with men. VIA is commonly used in low and middle-income countries (LMIC) achieving a moderate sensitivity with low specificity (31–33). In those areas with insufficient resources to manage large numbers of screen-positive women that would result from using a more sensitive but less specific HR-HPV DNA test, the OncoE6 test might be used for primary screening, thereby achieving a sensitivity similar or superior to VIA, which is already being widely used. However, because of its much higher specificity compared with VIA,

Table 2. The overall and site-specific percent test positive and prevalence of CIN grade 2 or more severe diagnosis (CIN2$^+$) and grade III or more severe diagnosis (CIN3$^+$)

	Percent test positive						Prevalence	
		*care*HPV		Hybrid capture 2				
	HPV E6 (HPV16/18/45)	Clinician	Self	Clinician	Self	VIA	CIN2+	CIN3+
Overall ($n = 7,421$)	1.8	14.4	14.5	14.5	17.9	7.3	1.9	1.3
By site								
Yangcheng ($n = 3,180$)	1.5	13.6	14.5	14.3	17.7	6.2	1.7	1.0
Xinmi ($n = 2,463$)	1.9	12.9	14.0	13.4	17.3	5.4	1.5	1.3
Tonggu ($n = 1,778$)	2.0	17.8	15.1	16.4	19.1	11.9	3.0	1.9
P^a	0.4	<0.001	0.6	0.02	0.3	<0.001	0.001	0.029

[a]Pearson χ^2 test.

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Table 3. Percent test positive by severity of diagnosis

		*care*HPV (HPV DNA)		HC2 (HPV DNA)		
	HPV E6 (HPV16/18/45)	Clinician	Self	Clinician	Self	VIA
Negative[a] ($n = 7,089$)	0.8	10.9	11.4	10.9	14.6	5.9
CIN1 ($n = 188$)	8.5	83.5	76.6	88.8	87.2	30.9
CIN2 ($n = 45$)	17.8	93.3	80.0	93.3	93.3	40.0
CIN3 ($n = 86$)	48.8	96.5	83.7	96.5	91.9	45.4
Cancer ($n = 13$)	84.6	100.0	84.6	100.0	84.6	84.6
<CIN2 ($n = 7,277$)	1.0	12.7	13.1	12.9	16.5	6.5
CIN2+ ($n = 144$)	42.4	95.8	82.6	95.8	91.7	47.2
<CIN3 ($n = 7,322$)	1.1	13.2	13.5	13.4	16.9	6.7
CIN3+ ($n = 99$)	53.5	97.0	83.8	97.0	90.9	50.5

NOTE: Clinician refers to clinician-collected cervical specimens; self refers to self-collected cervicovaginal specimens.
[a]Includes women who did not have biopsies and biopsies that were diagnosed as negative.

screening with HPV E6 might reduce either (i) the number of referrals to colposcopy when diagnostic verification is required, thereby saving on scarce clinical and financial resources or (ii) overtreatment in the context of a screen-and-treat program.

We noted that the percent E6-positives increased with increasing certainty of precancer and it was highest among those diagnosed with cancer. Even after controlling for the causal HPV genotypes, many CIN2 and CIN3 did not test E6 positive. This may not be surprising for an equivocal diagnosis of CIN2, which is more regressive and more likely to be caused by noncarcinogenic HPV genotypes than CIN3 (34–37), but it was more surprising for a CIN3 diagnosis, which to date is our best proxy for cervical precancer (i.e., cancer risk). Some of these misses were undoubtedly due to false-negative results.

We also speculate that not all CIN3 are equivalent in their invasive potential, with less than half of CIN3 lesions progressing to cancer if untreated after 30 years (38), as previously suggested (39). We hypothesize that the E6 expression levels may correlate with the oncogenic potential and progression from CIN3 to cancer. Perhaps, not surprisingly, we observed that the percent E6-positive (sensitivity) for HPV16/18/45 DNA-positive $CIN2^+$ (sensitivity) was nearly twice as great among $p16^{INK4a}$-immunohistochemistry positive versus negative tissues. Like E6, E7 is predicted to be overexpressed in precancerous tissue/transforming infections, which then interferes with retinoblastoma protein cell-cycle control pathway and leads to upregulation of $p16^{INK4a}$.

The clinical meaning and appropriate management of HPV E6-positive women without $CIN2^+$ are uncertain. Women who are HPV E6-positive are at high-risk of having CIN2, CIN3, or cancer and may suggest that aggressive management and treatment is appropriate, especially if multiple biopsy protocols are not used to maximize disease detection; notably, 31% of the HPV E6-positive $CIN2^+$ were diagnosed and the absolute risk of $CIN2^+$ was 29% among the colposcopically normal women (data not shown). The one-year follow-up of screen-positive patients in this study will provide some insight for the residual risk of undiagnosed $CIN2^+$ following a HPV E6-positive result.

We note that the sensitivity of OncoE6 for $CIN2^+$ and $CIN3^+$ increased at older ages, which may suggest that CIN3 develops invasive potential over time (39). This is consistent with our understanding of HPV natural history in which the median age of CIN2/3 occurs 10 to 15 years before the median age of invasive cervical cancer (40).

We found that the accuracy of HR-HPV DNA testing by HC2 and *care*HPV in this study to be as good as or better than previous reports from studies conducted in China: (i) the sensitivity and specificity for HC2 testing of clinician-collected specimens was similar to that reported in pooled analysis of data collected in China (41); (ii) the sensitivity and specificity for HC2 testing of self-collected specimens was better than reported in pooled analysis of data collected in China (42); and (iii) the sensitivity and specificity for *care*HPV testing of clinician-collected specimens and self-collected specimens was better than reported in the previous trial conducted China (11). The overall performance of the 2 tests on clinician-collected specimens was better than pooled analyses for HR-HPV DNA testing for primary cervical cancer screening (43). We cannot explain these differences. It is possible that differences in sensitivity were due to verification of disease in the screen-negative populations in this study versus previous studies. Differences in specificity are likely to be attributed to differences in prevalence of HR-HPV in populations sampled for this study versus the previous studies.

We acknowledge an important limitation with this study: we were restricted on what we could do for verification bias, primarily due to the projected lack of disease in this subgroup (44). Women who were screen negative and had no visible lesions could not be biopsied. We therefore may have overestimated the clinical sensitivity of the tests evaluated and the results are relative rather

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Table 4. The relationship of E6 and DNA detection of HPV genotypes 16, 18, and/or 45 (HPV16/18/45) on cervical exfoliated cells with HPV genotypes detected in the tissue of CIN2, CIN3, cervical cancer, CIN2+, and CIN3+ diagnoses

A. Worst, H&E diagnosis

	CIN2 ($n = 45$)					CIN3 ($n = 86$)					Cancer ($n = 13$)					CIN2+ ($n = 144$)					CIN3+ ($n = 99$)				
		E6		DNA			E6		DNA			E6		DNA			E6		DNA			E6		DNA	
	n	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)
HPV16+	14	6	42.86	14	100.0	61	40	65.57	56	91.8	12	11	91.67	12	100.0	87	57	65.52	82	94.25	73	51	69.86	68	93.15
HPV18+	5	2	40.0	4	80.0	0	0	0.0	0	0.0	0	0	0.0	0	0.0	5	2	40.0	4	80.0	0	0	0.0	0	0.0
HPV45+	0	0	0.0	0	0.0	1	1	100.0	1	10.0	0	0	0.0	0	0.0	1	1	100.0	1	100.0	1	1	100.0	1	100.0
HPV16/18/45+	19	8	42.11	18	94.74	62	41	66.13	57	91.94	12	11	91.67	12	100.0	93	60	64.52	87	93.55	74	52	70.27	69	93.24
Other carcinogenic	22	0	0.0	1	4.55	20	0	0.0	3	15.0	0	0	0.0	0	0.0	42	0	0.0	4	9.6	20	0	0.0	3	15.0
Noncarcinogenic	3	0	0.0	1	33.33	2	0	0.0	0	0.0	0	0	0.0	0	0.0	5	0	0.0	1	20.0	2	0	0.0	0	0.0
HPV Negative	1	0	0.0	1	100.0	2	1	50.0	2	100.0	1	0	0.0	0	0.0	4	1	25.0	3	75.0	3	1	33.33	2	66.67

B. p16-negative

	CIN2 ($n = 5$)					CIN3 ($n = 11$)					Cancer ($n = 0$)					CIN2+ ($n = 16$)					CIN3+ ($n = 11$)				
		E6		DNA			E6		DNA			E6		DNA			E6		DNA			E6		DNA	
	n	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)
HPV16+	1	0	0.0	1	100.0	7	3	42.86	6	85.71	0	0	n/a	0	n/a	8	3	37.5	7	87.5	7	3	42.86	6	85.71
HPV18+	0	0	0.0	0	0.0	0	0	0.0	0	0.0	0	0	n/a	0	n/a	0	0	0.0	0	0.0	0	0	0.0	0	0.0
HPV45+	0	0	0.0	0	0.0	0	0	0.0	0	0.0	0	0	n/a	0	n/a	0	0	0.0	0	0.0	0	0	0.0	0	0.0
HPV16/18/45+	1	0	0.0	1	100.0	7	3	42.86	6	85.71	0	0	n/a	0	n/a	8	3	37.5	7	87.5	7	3	42.86	6	85.71
Other carcinogenic	3	0	0.0	0	0.0	2	0	0.0	1	50.0	0	0	n/a	0	n/a	5	0	0.0	1	20.0	2	0	0.0	1	50.0
Noncarcinogenic	0	0	0.0	0	0.0	1	0	0.0	0	0.0	0	0	n/a	0	n/a	1	0	0.0	0	0.0	1	0	0.0	0	0.0
HPV Negative	1	0	0.0	1	100.0	1	1	100.0	1	100.0	0	0	n/a	0	n/a	2	1	50.0	2	100.0	1	1	100.0	1	100.0

C. p16-positive

	CIN2 ($n = 40$)					CIN3 ($n = 75$)					Cancer ($n = 13$)					CIN2+ ($n = 128$)					CIN3+ ($n = 88$)				
		E6		DNA			E6		DNA			E6		DNA			E6		DNA			E6		DNA	
	n	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	n (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)	*n*	*n* (+)	% (+)	*n* (+)	% (+)
HPV16+	13	6	46.15	13	100.0	54	37	68.52	50	92.59	12	11	91.67	12	100.0	79	54	68.35	75	94.94	66	48	72.73	62	93.94
HPV18+	5	2	40.0	4	80.0	0	0	0.0	0	0.0	0	0	0.0	0	0.0	5	2	40.0	4	80.0	0	0	0.0	0	0.0
HPV45+	0	0	0.0	0	0.0	1	1	100.0	1	100.0	0	0	0.0	0	0.0	1	1	100.0	1	100.0	1	1	100.0	1	100.0
HPV16/18/45+	18	8	44.44	17	94.44	55	38	69.09	51	92.73	12	11	91.67	12	100.0	85	57	67.06	80	94.12	67	49	73.13	63	94.03
Other carcinogenic	19	0	0.0	1	5.26	18	0	0.0	2	11.11	0	0	0.0	0	0.0	37	0	0.0	3	8.11	18	0	0.0	2	11.11
Noncarcinogenic	3	0	0.0	1	33.33	1	0	0.0	0	0.0	0	0	0.0	0	0.0	4	0	0.0	1	25.0	1	0	0.0	0	0.0
HPV Negative	0	0	0.0	0	0.0	1	0	0.0	1	100.0	1	0	0.0	0	0.0	2	0	0.0	1	50.0	2	0	0.0	1	50.0

[a]HPV16/18/45 E6 was detected by the AVantage HPV E6 test and HPV16/18/45 DNA was detected by a research-use only probe set developed for the *care*HPV platform and used only among the *care*HPV-positive women (data for the clinician-collected specimens only). The results of the HPV genotyping of the tissues were hierarchically categorized according to cancer risk to handle rare circumstances in which multiple HPV genotypes were detected: HPV16 > HPV18 > HPV45 > other carcinogenic HPV genotypes (HPV31, 33, 35, 39, 51, 52, 56, 58, 59, or 68) > noncarcinogenic HPV genotypes (including HPV positive with no HPV genotype identified)> HPV negative. The results are present for all diagnoses and stratified on whether the tissue tested $p16^{INK4a}$ positive by immunohistochemistry.

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Table 5. Sensitivity, specificity, PPV, and negative-predictive value (NPV) with 95% CIs of these tests for cervical intraepithelial neoplasia grade 2 or more severe ($CIN2^+$) diagnoses and grade 3 or more severe ($CIN3^+$) diagnoses

		*care*HPV		Hybrid Capture 2[a,b]		
	E6	Clinician[c]	Self[c]	Clinician[c]	Self[c]	VIA
CIN2 + (n = 144)						
Sensitivity	42.4 (34.2–50.9)	95.8 (91.2–98.5)	82.6 (75.4–88.4)	95.8 (91.2–98.5)	91.7 (85.9–95.6)	47.2 (38.9–55.7)
Specificity	99.1 (98.8–99.3)	87.3 (86.5–88.1)	86.9 (86.1–87.7)	87.1 (86.3–87.9)	83.6 (82.7–84.4)	93.6 (93.0–94.1)
PPV	46.9 (38.1–55.9)	13.0 (11.1–15.2)	11.1 (9.3–13.1)	12.9 (10.9–15.0)	10.0 (8.4–11.7)	12.7 (10.0–15.9)
NPV	98.86 (98.59–99.09)	99.91 (99.79–99.97)	99.61 (99.42–99.74)	99.91 (99.79–99.97)	99.80 (99.66–99.90)	98.90 (98.6–99.13)
CIN3 + (n = 99)						
Sensitivity	53.5 (43.2–63.6)	97.0 (91.4–99.4)	83.8 (75.1–90.5)	97.0 (91.4–95.8)	90.9 (83.4–95.8)	50.5 (40.3–60.7)
Specificity	98.9 (98.7–99.2)	86.8 (86.0–87.6)	86.5 (85.7–87.2)	86.6 (85.8–87.4)	83.1 (82.2–83.9)	93.4 (92.8–93.9)
PPV	40.8 (32.2–49.7)	9.1 (7.4–10.9)	7.7 (6.2–9.5)	9.0 (7.3–10.8)	6.8 (5.5–8.3)	9.4 (7.0–12.2)
NPV	99.37 (99.16–99.54)	99.95 (99.86–99.99)	99.75 (99.59–99.86)	99.95 (99.86–99.99)	99.85 (99.72–99.93)	99.29 (99.06–99.47)

Abbreviations: E6, AVantage HPV E6 test for HPV16, 18, and 45.

[a]We used a research-use only protocol for HC2 to test CCM specimens, which may have led to a decrement of clinical performance.

[b]We note that HC2 using this protocol had a very similar sensitivity and specificity for CIN2+ and CIN3+, as was reported for pooled results of HC2 from 17 studies conducted in China (44).

[c]Clinician refers to clinician-collected cervical specimens; Self refers to self-collected cervicovaginal specimens.

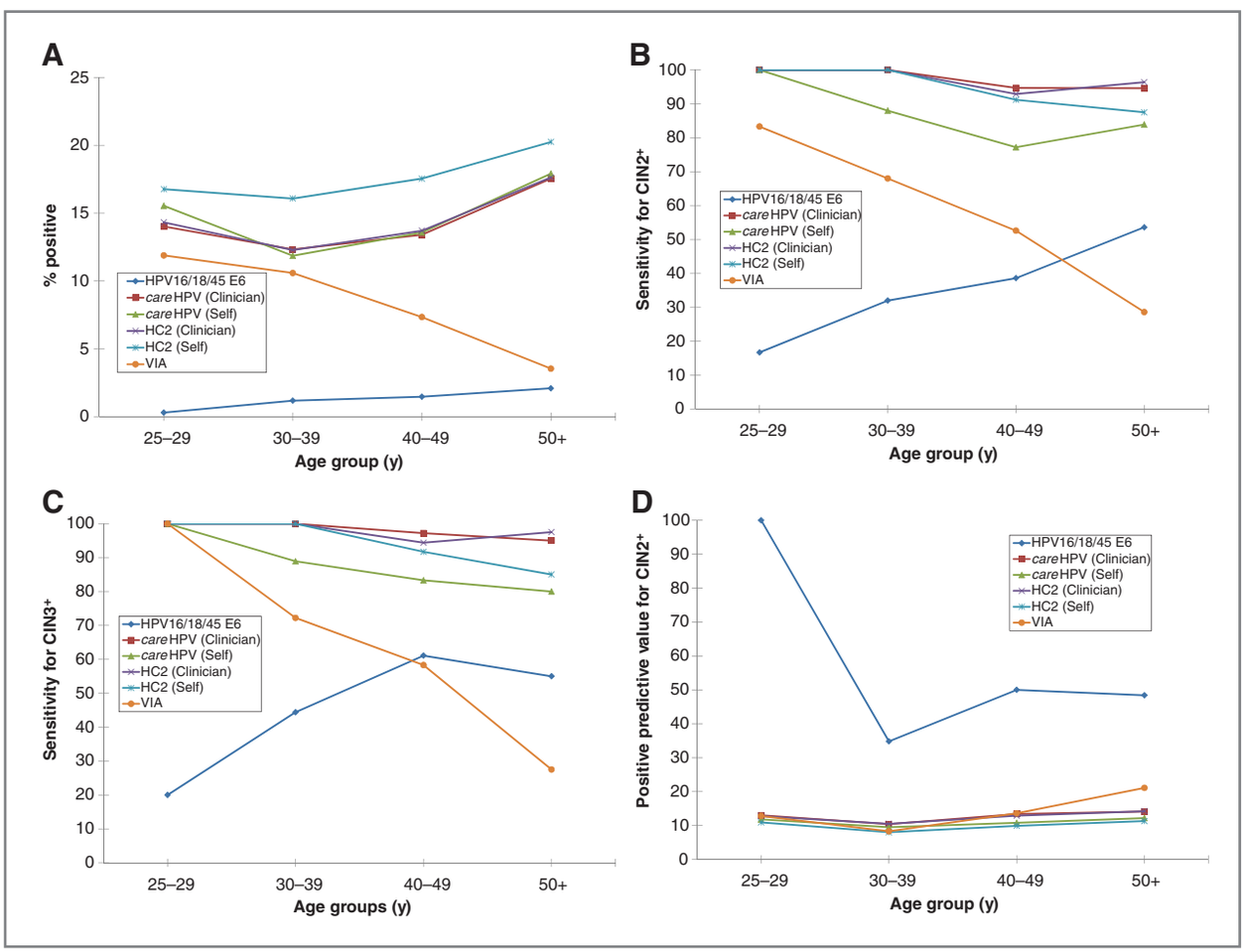

Figure 2. The percentage of positive among all women (A), women with CIN2, or more severe diagnosis ($CIN2^+$; sensitivity; B), and grade 3 (CIN3) or more severe diagnosis ($CIN3^+$; C) and the positive predictive value for $CIN2^+$ (D) by age group. —◆—, HPV16/18/45 E6; —■—, *care*HPV (Clinician); —▲—, *care*HPV (Self); —×—, HC2 (Clinician); —✳—, HC2 (Self); —●—, VIA.

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than absolute. However, we highlight the fact that with 2 HR-HPV DNA tests conducted on 2 samples, one OncoE6 and VIA, it is highly unlikely that any women with CIN2$^+$ were not sent to colposcopy.

In conclusion, we conducted a comprehensive study of screening tools that might be used in LMICs where to date cytology-based programs have largely failed. We confirmed the comparability of the Chinese State Food and Drug Administration-approved *care*HPV to HC2 (11), which has also been made available via tiered pricing to some LMICs (45). We provided the first population-based data for a promising new E6 oncoprotein test that might be useful in high HPV prevalence populations and as a triage test for HPV-positive women. The choice of screening tests and algorithms will depend on resources, balancing of the benefits and harms of screening (46), and the acceptable cancer risk (47). Future research should focus on practical application of these screening tools, i.e., validation of screening and management algorithms in real-world settings, as they would be used for implementation at the regional or national level. Such translation of these tools will accelerate the reduction of the cervical cancer burden now while we wait for prophylactic HPV vaccines to reduce the population risk in the future.

Disclosure of Potential Conflicts of Interest

J. Schweizer and P. Lu have ownership interests (including patents) in Arbor Vita Corp. R. Peck has a commercial research grant in Arbor Vita Corporation SBIR phase II grant—PATH is a subrecipient of funding. M.H. Stoler is a consultant/advisory board member of Merck, Roche/Ventana, BD, Hologic/GenProbea, and Qiagen. P.E. Castle has honoraria from speakers' bureau in Roche, is a consultant/advisory board member of GE Healthcare, BD, Roche, Merck, and Cepheid. No potential conflicts of interest were disclosed by the other authors.

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