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Comparison of primary cytology, primary HPV testing and co-testing as cervical cancer screening for Chinese women: a population-based screening cohort

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ABSTRACT

Objectives We compared clinical performance of three strategies of primary human papillomavirus (HPV) testing, primary cytology and co-testing for cervical cancer screening.

Design A population-based prospective cohort study of clinical performance of screening strategy.

Setting Patients recruited from community in Changzhi County, Shanxi Province, China.

Patient 3209 women aged 30–64 years without gynaecological issues.

Primary and secondary outcome measures The performance of different screening strategies for detecting cervical intraepithelial neoplasia grade 2 or more severe (CIN2+).

Results A total of 53 CIN2+ and 31 CIN3+ cases are detected. For CIN2+, sensitivity of primary HPV (95.9%) and co-testing (98.0%) are not statistically different, but significantly higher than primary cytology (48.0%). Specificity (86.8%), colposcopy referral rate (7.8%) and number of colposcopies required to detect one case (9.8) for primary HPV are better than co-testing (79.8%, 11.9%, 14.3%, respectively). For CIN3+, primary HPV, co-testing have 100% of sensitivity and specificity, which is significantly higher than primary cytology (56.7% and 90.2%). Number of colposcopies required to detect one case for primary HPV (15.9) is better than co-testing (23.8).

Conclusions Compared with co-testing, HPV primary screening had comparable sensitivity and higher specificity for CIN2+ detection, and both of them showed better performance than cytology primary screening in cervical cancer screening.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ From 2018 to 2020, the low loss to follow-up rate (11.9%) increase the stability of our results.
⇒ A rigorous diagnostic procedure performed by cytologists and pathologists (a diagnostic panel of three senior doctors) ensure the accuracy of the end point.
⇒ The single-centre study limits the extrapolation of our results.
⇒ Three strategies based on one population might have cross-contamination, and rigorous randomised controlled trial is preferred.
⇒ Although results of cytology and pathology have a strict quality control process, human papillomavirus testing is based on only one company's kit which may be biased.

INTRODUCTION

In May 2018, WHO made a global call for action towards the elimination of cervical cancer, which was to be achieved through human papillomavirus (HPV) vaccination, and effective cervical cancer screening, treatment and management.[1] Disparities in economic development and uneven distribution of health resources threaten the progress of cervical cancer elimination in China.[2] In China, the HPV vaccine was approved for marketing in 2016, a decade later than the earliest country to market it. A survey showed that the HPV vaccination rate for girls aged 9–14 years in China was <1% in 2020.[3] The proposal of the National Committee of the Chinese People's Political Consultative Conference in 2020 suggested that HPV vaccine should be included in the national immunisation programme (NIP). As of now, cities such as Erdos and Xiamen have started free HPV vaccination for school-aged girls. Then, 112 countries have included HPV vaccine in NIP as of 2021, but China has not yet done so; therefore, the HPV vaccination rate of Chinese school-aged girls is much lower than that of high-income countries with NIP.[4]

In 2004, The Guidelines for Cervical Cancer Screening and Early Diagnosis and
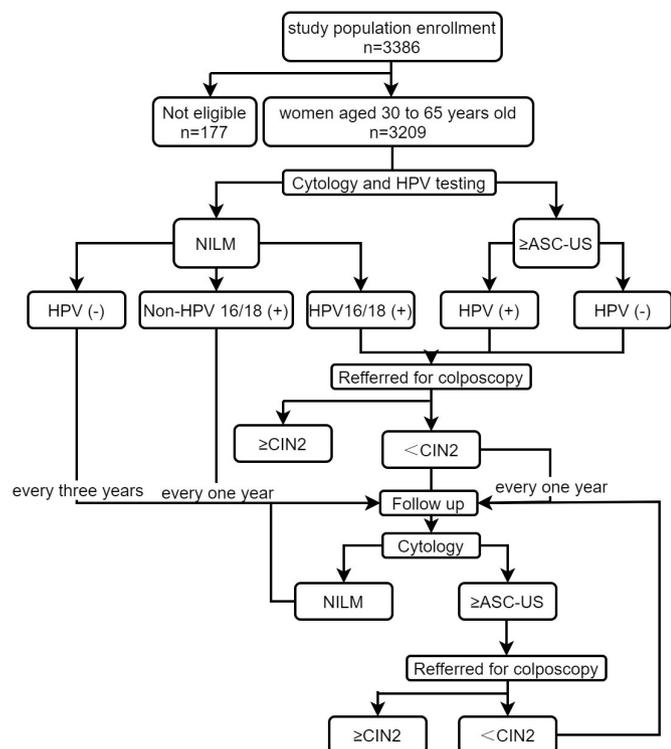
Figure 1 Participant flow of enrolment in the screening cohort. ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy.

Treatment in China was developed to guide the practice of cervical cancer prevention and treatment strategies for the cervical cancer population. Then in 2005, the 'National Cervical Cancer Early Diagnosis and Treatment Demonstration Bases' were established in Shenzhen and Xiang Yuan County in urban and rural areas, respectively. From 2006 to 2008, 43 regions in 31 provinces nationwide were supported to carry out cervical cancer prevention and treatment.[5] At the initial stage of the cervical cancer screening programme, the detection methods used were highly subjective and poorly sensitive, so that the problem of misdiagnosis, missed diagnosis or repeated screening was serious, with an average annual screening of 3.8 million people and low population coverage. From 2009 to 2018, the total number of cervical cancer screenings exceeded 85 million.

In addition, in 2015, a cross-sectional survey was conducted in 298 districts/counties across 31 provincial-level administration divisions (PLADs), which were selected randomly from over 2400 districts/counties to be representative of the whole population in the Mainland China as well as the population in every PLAD and specific regions, including urban and rural areas.[6] Results showed that provincial-level data further showed that screening rates varied widely across the 31 PLADs. The screening rates in five provinces, including Beijing and Shanghai, exceeded 35%, whereas three provinces, Tibet, Anhui and Hebei, had screening rates of <15%.

To achieve the WHO's goal that 70% of women be screened, a sustainable cervical cancer screening strategy should be adapted to improve screening coverage in the real world.[1] Cytology had been the primary method for screening in the past decades. However, growing evidence have shown that inclusion of HPV testing alone or combined with cytology (co-testing) for screening, compared with cytology alone, is associated with a subsequent reduction in precancerous lesions. Cytology is affordable and is easily adapted to different settings, but its accuracy depends largely on the cytologist.[7 8] On the other hand, HPV DNA testing is often preferred for its high accuracy and reproducibility. However, strict environmental and equipment requirements have limited its usage in low-income and middle-income countries (LMICs).[9–11] Furthermore, the majority of HPV infection will be cleaned within 6–24 months in women even without any intervention.[12 13] Therefore, screening with HPV testing alone could result in overdiagnosis, because HPV-positive individuals without the risk of cervical cancer may seek treatment.[14 15] A combination of HPV DNA and cytology (named 'co-testing') could greatly improve clinical accuracy, as it combines the high specificity of cytology testing and high sensitivity of HPV testing, but co-testing is always along with higher costs.

According to the clinical performance of the test and the resource availability of the area, different sustainable screening strategies should be tailored in China. Therefore, in this prospective screening cohort, we compare clinical performance of stand-alone and co-testing strategies with cytology and HPV tests in one population.

METHODS

Participants recruitment

The Changzhi County, Shanxi Province is an area with a high prevalence of HPV infection[16–18] and with low health and economic resource. The 10 townships were divided into 3 clusters based on geographical location, and then 1 township was randomly selected from each cluster. A total of 3386 women without gynaecological issues were involved at baseline. The inclusion criteria were as follows: (1) the patients were 30–64 years old with intact uterus; (2) there was no history of cervical cancer treatment or cervical surgery; (3) after screening, sexual life was prohibited within 48 hours, and no vaginal medication, vaginal contraceptives or vaginal lotion were found within 48 hours; (4) women without suspected clinical pregnancy symptoms; pregnant women could participate in the study at 8 weeks after the end of pregnancy; (5) informed consent was signed, cytology or biopsy samples were collected for testing. Exclusion criteria: those who do not meet any of the above inclusion criteria. Finally, 177 women were excluded and 3209 women were tested by both cytology and HPV DNA at baseline (figure 1). All women with positive baseline result were followed up for 3 years, and in the last year, all participants were also tested by both HPV and cytology.

Sample size calculation

According to our previous research (data are not shown), the cumulative incidence of cervical intraepithelial neoplasia grade 2 or more severe (≥CIN2) at 3 years in a population with normal baseline cytology ranged from approximately 1.13% to 2.1%. We hypothesise that the number of cases with ≥CIN2 on pathology at the end of follow-up should be no less than 20. Therefore, the cumulative incidence of ≥CIN2 at 3 years combined with the above-mentioned cumulative incidence of ≥CIN2, it is estimated that the population requiring NILM is 20/1.13%=1770 cases. The total sample size required was estimated based on the proportion of NILM in the total cervical cancer screening population. According to our previous research (data are not shown), the percentage of cervical NILM in Chinese women is about 83% of the total population, and considering a 30% missed follow-up rate, about 2772 women need to be recruited for cervical cancer screening. Considering other factors, to ensure a sufficient population and number of cases required for combined screening in the total screening population, at least 3000 women were planned to be included in this study.

Three screening strategies

All 3209 women were tested by both cytology and HPV DNA at baseline and follow-up for 3 years by cytology. In the last year, all participants were also test by both HPV and cytology. According to the Chinese Guidelines for Cervical Cancer Screening,[19] three sets of data were extracted from one population in our study to evaluate the clinical performance of three screening strategy.

In strategy 1 (primary HPV with triage by 16/18 genotyping and cytology), participants were primarily screened with HPV testing. Those tested positive for HPV 16/18 were referred to colposcopy, and the 12 other high-risk (HR)-HPV(+) were triaged with cytology. Among them, those diagnosed with ≥ASC-US were referred to colposcopy.

In strategy 2 (primary cytology with triage by HPV 16/18 genotyping), participants were primarily screened with cytology alone. Among them, those diagnosed with>ASC-US were referred to colposcopy. ASC-US were triaged with reflex HPV testing, and HPV 16/18+ were referred to colposcopy.

In strategy 3 (co-testing with genotyping and cytology triage: HPV 16/18 and ASC-US HPV+ threshold) women were screened by both cytology and HPV testing, and those with either HPV 16/18 +, or >ASC-US, or ASC-US and HPV+ were referred to colposcopy. More details are shown in figure 2. Notably, three strategies were conducted in one same population-based cohort, but data were analysed by different strategies.

Sample collection

All patients first underwent gynaecological examinations. Cervical exfoliated cells were collected using a cytology brush (Hologic, Bedford, Massachusetts, USA) and stored in the tubes with preservation solution for the Thinprep cytology test (Hologic). A 2.5 mL PreservCyt Liquid was separated from each cytological specimen and placed in a special specimen tube for HPV DNA test (Tegen, Shanghai). The remaining PreservCyt Liquid was subjected to cytological examination.

Laboratory tests

All of the following tests and diagnostic procedures were strictly double-blinded. Cytology slides were read by two pathologists results and reported according to the Bethesda 2014 classification. The cytological results were as follows: negative for intraepithelial lesion or malignancy (NILM), atypical cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells and cervical cancer cells. Diagnoses were reported if the diagnoses by two cytologists were consistent. Otherwise, a third cytologist was consulted.

A commercial assay was used for HPV DNA testing. And HPV testing method was Biochip Method, the manufacturer was Beijing Bohui Innovative Optoelectronic Technology and the China Food and Drug Administration (CFDA) approval number was obtained (registration certificate n0: 20163401108). The results were categorised as following: HPV−, HR-HPV (result positive for 1 or more of 14 HR types (HPV 16, HPV 18, HPV 31, HPV 33,HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66 and HPV 68)), HPV 16/18+ (result positive for either genotype 16 or 18) and HPV non-16/18+ (result negative for genotype 16/18 and positive for 1 or more of 12 other HR types).

Final screening assessment

All women with positive HPV 18 and/or HPV 16 testing or abnormal cytology (ASC-US or worse) were referred to colposcopy. If the colposcopy is fully exposed and the lesion site exists, biopsy should be carried out in the abnormal part, and the specific location of the specimen should be clearly marked; if the colposcopy exposure is insufficient, cervical curettage should be performed. Two pathologists independently made diagnosis according to the 2014 WHO Classification of Tumours of the Female Genital Tract. If the diagnoses were concordant, they were reported as the pathological diagnosis. Otherwise, two pathologists will read all cytology slides, and for all positive results and 10% of negative results, a third pathologist will read the slides and then take the results that are in agreement between the three doctors, and for the results that are not in agreement a decision will be made by all three. All technicians, cytologist and pathologist involved in HPV testing and cytology slides reading were double-blind in the whole process of our study. The histological diagnoses of cervical lesions were divided into normal, LSIL/CIN1 (including the condylomatous variant), HSIL/CIN2, HSIL/CIN3 (including adenocarcinoma in
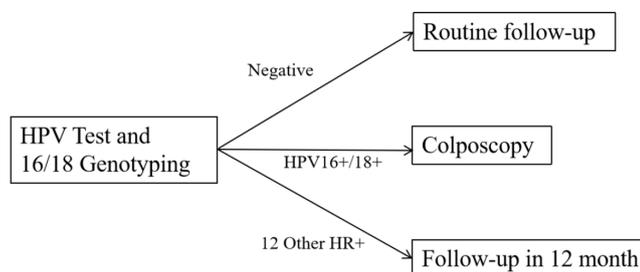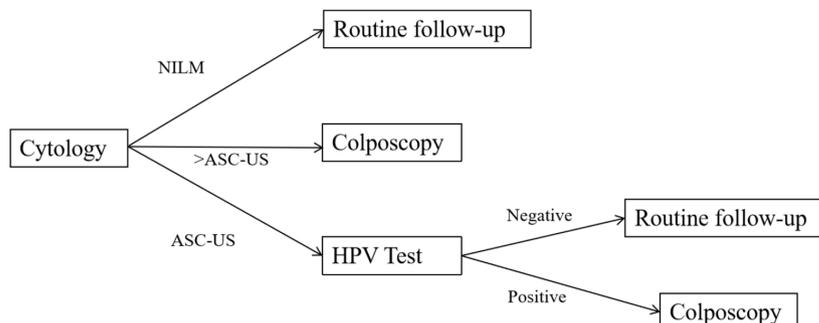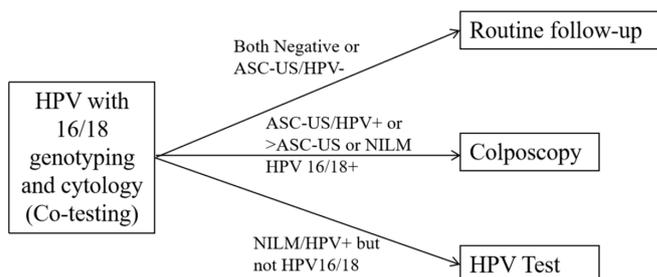
Strategy 1: Primary HPV with triage by 16/18 genotyping and cytology: ASC-US threshold

Strategy 2: Cytology with triage by HPV 16/18 genotyping

Strategy 3: Co-testing with genotyping and cytology triage: HPV 16/18 & ASC-US HPV+ threshold

Figure 2 Screening strategies in this population-based cohort. Strategy 1: primary HPV with triage by 16/18 genotyping and cytology: ASC-US threshold. Strategy 2: cytology with triage by HPV 16/18 genotyping. Strategy 3: co-testing with genotyping and cytology triage: HPV 16/18 and ASC-US HPV+ threshold. ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR, high-risk; NILM, negative for intraepithelial lesion or malignancy.

situ) and carcinoma (squamous cell carcinoma or adenocarcinoma). HPV testing, cytology and pathological examination were performed with blinding to results of each test. Women with both negative HPV and cytology results were not referred to colposcopy and regarded as normal.

Follow-up and end point

The end point is histologically confirmed ≥CIN2. At baseline, those with positive HPV 18 and/or HPV 16 testing or abnormal cytology (ASC-US or worse) were immediately referred to colposcopy, and pathological results below CIN2 would be followed up with cytology every year. Those with cytology (NILM) and non-HPV 16/18 (+) would be followed up with cytology every year; those with normal cytology (NILM) and HPV(−) would be followed up with cytology every 3 years. More details are shown in figure 1.

At the end of follow-up, all women were screened with cytology and HPV DNA testing.

Statistical analysis

In this study, pathological diagnosis results were viewed as 'Golden Criteria' to compare three screening strategies. In addition, for those with negative screening results at baseline and third year, they would not be clinically recommended to conduct colposcopy due to the low prevalence rate of cervical lesions,[20] therefore they were viewed as normal pathological results in final statistical analysis. The clinical performance of the three strategies includes accuracy and cost of screening technology, the accuracy of detecting high-grade CIN is first calculated using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), colposcopy referral rate and number needed to refer (NNR) to colposcopy to find one case of CIN2+ or CIN3+. And

95% CIs for proportions were calculated according to the efficient-score method (corrected for continuity) described by Robert Newcombe, based on the procedure outlined by Wilson.[21 22] Second, the cost of each strategy was also calculated in the real world, and the standard price for each screening strategy from the National Rural-Area Two-Cancer Screening Project was used: 35 RMB for a cytology examination, 112 RMB for a HPV DNA test and 220 RMB for a histopathological biopsy. Three screening strategies were from the same one cohort, therefore participants would referred to colposcopy if any condition in any screening strategy was satisfied. In cases where a participant was diagnosed with CIN2+ by one screening strategy at baseline, while the other strategy determined that follow-up was needed, and the participant would be viewed as end point, therefore it is unclear whether other strategy could detect CIN2+ in the following years. Sensitivity analysis was conducted to improve the stability of the results. SAS statistical software (V.9.4) was used for the statistical analyses.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research. However, patient groups were involved in approving the patient information leaflet for sample collection.

RESULTS

In 2017, a total of 3209 women were screened and 687 women with positive baseline results were needed to follow-up for 3 years. From 2018 to 2020, 18.9% (130/687) of participants were lost to follow-up. In 2020, all participants were recalled and compliance rate was 88.1% (2932/3328). In this screening cohort, a total of 157 cases of CIN1, 53 cases of CIN2 or worse (CIN2+) and 31 cases of CIN3 or worse (CIN3+) were detected, and population characteristics are shown in online supplemental table 1. In our cohort, 77, 68 and 130 participants were lost to follow-up in strategy 1, 2 and 3, respectively. Strategy 1 has detected 47 cases of CIN2+ and 29 cases of CIN3+, 24 cases of CIN2+ and 17 cases of CIN3+ in strategy 2, 49 cases of CIN2+ and 29 cases of CIN3+ in strategy 3. Four cases were indicated as needing follow-up but diagnosed by other strategies, four, two and three cases in strategy 1,

2 and 3. More details are shown in online supplemental table 2.

Distribution of HPV and cytological diagnosis among general population at baseline

Among all screened participants, 13.9% (446/3209) of women had abnormal cytology, 14.4% (463/3209) were HR-HPV positive and 4.7% (152/3209) were HPV 16/18 positive. The abnormal rate of cytology was highest in people aged 60–64 years (38.9%), followed by 50–59 years (21.8%), 40–49 years (9.7%) and 30–39 years (6.2%). The prevalence of HR-HPV peaked at age 60–64 years (17.9%), followed by 50–59 years (16.7%), 30–39 years (14.5%) and 40–49 years (12.6%). The positive rate of HPV16/18 was 6.2% in the 60–64 years group, 5.0% in 30–39 years group, 4.9% in 50–59 years group and 4.4% in 40–49 years group (table 1).

Distribution of HR-HPV and cytological diagnosis among different grades of cervical lesions at baseline

A total of 17.4% (558/3209) of the participants were referred to colposcopy. Among them, 5.2% (166/3209) were pathologically abnormal, including 128 cases with CIN1, and 38 with CIN2+. HR-HPV infection prevalence was 38.0% (212/558): 59.4% (126/212) with normal pathology and 40.6% (86/212) with abnormal pathology. Among those with CIN2+, only 5.3% (2/38) was not infected with HR-HPV and the most prevalent subtype was HPV 16 (72.2%), followed by HPV 18 (16.7%), HPV 58 (16.7%), HPV 52 (13.9%) and HPV 33 (11.1%). The total abnormal cytology rate was 75.4% (421/558): 70.1% (295/421) with normal pathology and 29.9% (126/421) with abnormal pathology. Among those with CIN2+, 55.3% (21/38) were diagnosed with ASC-US+ and 44.7% (17/38) with NILM. There was no correlation between the severity of cytological diagnosis and the grade of cervical lesions (table 2).

Comparison among three screening strategies to identify high-grade CIN with 3 years follow-up

When identifying CIN2+ as end point, strategy 1 had sensitivity rate of 95.9% and specificity rate of 86.8%, PPV of 10.2%, NPV of 99.9%, its colposcopy referral rate was 7.8% and the needed number of performed colposcopies

Table 1 Distribution of HPV and cytological diagnosis at different age groups at baseline

Age group (years)	Cytological diagnosis		HPV infection		HR-HPV infection		
	NILM (%)	≥ASC-US (%)	Non-HR-HPV (%)	HR-HPV (%)	16/18 (%)	12OT (%)	Total (%)
30–39	636 (93.8)	42 (6.2)	580 (85.5)	98 (14.5)	34 (5.0)	64 (9.4)	678 (100.0)
40–49	1308 (90.3)	140 (9.7)	1266 (87.4)	182 (12.6)	63 (4.4)	119 (8.2)	1448 (100.0)
50–59	720 (78.2)	201 (21.8)	767 (83.3)	154 (16.7)	45 (4.9)	109 (11.8)	921 (100.0)
60–64	99 (61.1)	63 (38.9)	133 (82.1)	29 (17.9)	10 (6.2)	19 (11.7)	162 (100.0)
Total	2763 (86.1)	446 (13.9)	2746 (85.6)	463 (14.4)	152 (4.7)	311 (9.7)	3209 (100.0)

12OT: HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66 and HPV 68.
ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR, high-risk; NILM, negative for intraepithelial lesion or malignancy; OT, other HR-HPV genotype.

Table 2 Results of HR-HPV and cytological diagnosis among different grades of cervical lesions at baseline

	Pathological diagnosis			
	Normal (%)	CIN1 (%)	CIN2+ (%)	Total (%)
HPV infection*				
Non-HR-HPV	266 (76.9)	78 (22.5)	2 (0.6)	346 (100.0)
HR-HPV	126 (59.4)	50 (23.6)	36 (17.0)	212 (100.0)
HPV 16/18	78 (57.3)	28 (20.6)	30 (22.1)	136 (100.0)
HPV 16	52 (53.1)	20 (20.4)	26 (26.5)	98 (100.0)
HPV 18	26 (65.0)	8 (20.0)	6 (15.0)	40 (100.0)
HPV 52	18 (50.0)	13 (36.1)	5 (13.9)	36 (100.0)
HPV 58	19 (63.3)	5 (16.7)	6 (20.0)	30 (100.0)
HPV 33	7 (53.8)	2 (15.4)	4 (30.8)	13 (100.0)
Cytological diagnosis				
NILM	97 (70.8)	23 (16.8)	17 (12.4)	137 (100.0)
ASC-US	126 (90.0)	10 (7.1)	4 (2.9)	140 (100.0)
AGC	11 (73.3)	4 (26.7)	0 (0.0)	15 (100.0)
LSIL	102 (68.0)	45 (30.0)	3 (2.0)	150 (100.0)
ASC-H	34 (44.7)	34 (44.7)	8 (10.5)	76 (100.0)
HSIL	22 (55.0)	12 (30.0)	6 (15.0)	40 (100.0)
Total	392 (70.3)	128 (22.9)	38 (6.8)	558 (100.0)

*Multiple infection was counted by each type.
AGC, atypical glandular cells; ASC-H, high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.

to detect one case was 9.8. Strategy 2 had the highest specificity (90.4%) and colposcopy referral rate was 9.4%, but it had the lowest sensitivity (48.0%) and NPV (99.1%). Strategy 3 showed the highest sensitivity (98.0%), NPV (99.9%) and colposcopy referral rate (11.9%), but it had the lowest specificity (79.8%) and PPV (7.0%). The

needed number of performed colposcopies to detect one case was 14.3 (table 3).

When CIN3+ as end point, both strategy 1 and strategy 3 had 100% of sensitivity rate and NPV, but strategy 1 had a higher specificity (86.4% vs 79.3%) and a higher PPV (6.3% vs 4.2%) than strategy 3. In addition, strategy 1 had a lower needed number of performed colposcopies to detect one case (15.9) than strategy 3 (23.8). Strategy 2 had the lowest sensitivity (56.7%) and NPV (99.5%), while it had the highest specificity rate (90.2%) and the needed number of performed colposcopies to detect one case was 19.2 (table 3). Moreover, sensitivity analysis was conducted to test the stability of the results. The adjusted results are shown in table 4, which show similar results to those in table 3.

DISCUSSION

According to the guidelines from the USA and Europe, individuals were recommended to start cervical cancer screening at 25 years old and undergo primary HPV testing every 5 years through 65 years old (preferred). However, the guideline in China recommended HPV or cytology alone or both strategies for women aged 30–64 years, but there is no preferred strategy.[6 23 24] This real-world study combines the baseline and the 3-year follow-up data, and provides evidence on the clinical performance of the three strategies with consideration of the cost of screening examination technology. Our results showed that HPV primary screening for women aged 30–64 years appears to be the optimal strategy.

Overall, HR-HPV prevalence in the study population at baseline was 14.4%, which was lower than 18.0%, as concluded by pooled analysis by Zhao *et al* from 17 population-based studies in rural China in 2010.[25] In our study, the prevalence of HPV slightly declined in middle

Table 3 Clinical performance and cost analysis of different screening strategies for CIN2+ and CIN3+ diagnoses after 3 years follow-up

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Colposcopy referral rate (%)	NNR*
≥CIN2						
Strategy 1	95.9 (90.1 to 100.0)	86.8 (85.7 to 88.0)	10.2 (9.2 to 11.2)	99.9 (99.7 to 100.0)	7.8 (6.8 to 8.7)	9.8 (8.9 to 10.9)
Strategy 2	48.0 (34.2 to 61.8)	90.4 (89.3 to 91.4)	7.3 (5.5 to 9.7)	99.1 (98.8 to 99.3)	9.4 (8.4 to 10.4)	13.7 (10.3 to 18.2)
Strategy 3	98.0 (94.0 to 100.0)	79.8 (78.4 to 81.2)	7.0 (6.5 to 7.5)	99.9 (99.7 to 100.0)	11.9 (10.8 to 13.0)	14.3 (13.3 to 15.4)
≥CIN3						
Strategy 1	100.0 (100.0 to 100.0)	86.4 (85.2 to 87.5)	6.3 (5.8 to 6.8)	100.0 (100.0 to 100.0)	7.8 (6.8 to 8.7)	15.9 (14.7 to 17.2)
Strategy 2	56.7 (38.9 to 74.4)	90.2 (89.2 to 91.2)	5.2 (3.8 to 7.1)	99.5 (99.3 to 99.7)	9.4 (8.4 to 10.4)	19.2 (14.1 to 26.3)
Strategy 3	100.0 (100.0 to 100.0)	79.3 (77.9 to 80.7)	4.2 (4.0 to 4.5)	100.0 (100.0 to 100.0)	11.9 (10.8 to 13.0)	23.8 (22.2 to 25.0)

CIN, cervical intraepithelial neoplasia; NNR, the number of performed colposcopies to detect one case; NPV, negative predictive value; PPV, positive predictive value.

Table 4 Clinical performance and cost analysis of different screening strategies for CIN2+ and CIN3+ diagnoses after 3 years follow-up after adjustment

	Adjusted 1				Adjusted 2			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥CIN2								
Strategy 1	88.7 (80.1 to 97.2)	86.8 (85.6 to 88.0)	10.2 (9.0 to 11.4)	99.8 (99.5 to 99.9)	96.2 (91.1 to 100)	86.9 (85.8 to 88.1)	11.0 (10.0 to 12.1)	99.9 (99.7 to 99.9)
Strategy 2	46.2 (32.6 to 59.7)	90.1 (89.3 to 91.4)	7.3 (5.5 to 9.7)	99.0 (98.8 to 99.2)	50.0 (36.4 to 63.4)	90.4 (89.4 to 91.5)	7.9 (6.0 to 10.3)	99.1 (98.8 to 99.3)
Strategy 3	92.3 (85.1 to 99.6)	79.8 (78.4 to 81.2)	7.0 (6.3 to 7.7)	99.8 (99.6 to 99.9)	98.1 (94.3 to 100.0)	79.9 (78.5 to 81.3)	7.4 (6.9 to 8.0)	99.9 (99.7 to 99.9)
≥CIN3								
Strategy 1	93.5 (84.9 to 100.0)	86.3 (85.1 to 87.5)	6.3 (5.6 to 7.1)	99.9 (99.7 to 100.0)	100.0 (100.0 to 100.0)	86.4 (85.2 to 87.6)	6.7 (6.2 to 7.3)	100.0 (100.0 to 100.0)
Strategy 2	54.8 (37.3 to 72.4)	90.2 (89.2 to 91.2)	5.2 (3.8 to 7.1)	99.5 (99.3 to 99.7)	58.1 (40.7 to 75.4)	90.2 (89.2 to 91.3)	5.5 (4.1 to 7.4)	99.5 (99.3 to 99.7)
Strategy 3	93.5 (84.9 to 100.0)	79.3 (77.9 to 80.7)	4.2 (3.8 to 4.7)	99.9 (99.7 to 100.0)	100.0 (100.0 to 100.0)	79.4 (78.0 to 80.8)	4.5 (4.2 to 4.8)	100.0 (100.0 to 100.0)

Adjusted 1: all cases found by other strategies were considered as missed cases. Adjusted 2: all cases found by other strategies were considered as detected cases.
CIN, cervical intraepithelial neoplasia; NPV, negative predictive value; PPV, positive predictive value.

ages (40–49 years), yet increased among the oldest people (60–64 years), which are consistent with other studies in China[26 27] or in some Latin America/Caribbean populations.[28] A meta-analysis of 1 million women from five continents showed that age-specific HPV distribution presented with a first peak at younger ages (<25 years) and, in the Americas and Africa, a rebound at older ages (≥45 years).[29] Such a bimodal pattern could be due to changes in the sexual behaviour or the reactivation of latent viral infections.[30] However, studies have shown that the relationship between HPV prevalence and age factors varied by regions. It seems that in Southern Europe, Western Africa, South America, the HPV prevalence shows a decreased trend before about 50 years, then it has an increased trend among 50 years to 65 years.[4 6] Compared with some studies in China,[26 27 31–33] the 'V' trend of HPV prevalence among age groups is more common among the Chinese population. Moreover, the prevalence of ≥ASC-US is also lower at 13.9% compared with 17.0%, as concluded from a pooled analysis of 13 population-based studies on rural women throughout China.[34] Notably, the observed rate of ASC-US+ in China is very high, which was higher than other countries, including 6.7% for the USA, 5.47% for Italy, 3.57% for Switzerland, 9.60% for France. In additoin, the lower rate of HR-HPV prevalence and cytological abnormality rate could potentially be explained by the cervical cancer screening and promotion campaigns in this area throughout the past 20 years.[35 36] In addition, we found that among women with CIN2+, the most frequent HPV types were 16, 58, 18 and 52, which was consistent with the cross-sectional population-based study in rural Northern China.[37]

For women aged 30–64 years, primary HPV with triage by 16/18 genotyping and cytology (strategy 1) is cost-effective, compared with co-testing, as shown in other studies as well.[38–40] To our knowledge, there is no evidence that co-testing can significantly improve the accuracy of screening than primary HPV. The main difference between primary HPV and co-testing is that in co-testing, women who are HPV(−) are triaged with cytology, whereas the triage does not occur in primary HPV.[41–43] When cytologists are inadequately trained, co-testing, compared with primary HPV, has similar accuracy, and worse colposcopy rate and NNR. For CIN2+, its specificity, PPV and NPV are much higher, its colposcopy referral rate and NNR are lower, although its sensitivity is lower (not statistically significant). Because participants of screening are generally healthy people, it is of great public health significance to reduce unnecessary examination and psychological anxiety related to overdiagnosis, which is reflected by colposcopy referral rate and NNR.[44] In addition, it is possible for individual with CIN2 to return to normal,[12 39 40] thus we also consider CIN3+ to be the disease end point. For CIN3+, we found all accuracy indicators of primary HPV to be equal to or better than co-testing.

Although the total and annual costs of cytology for all participants are only one-third of the costs of co-testing, the accuracy of cytology combined with triage by HPV testing (strategy 2) was far less than the accuracy of co-testing and primary HPV, which is already well-established in other studies.[45 46] Furthermore, our accuracy of cytology is lower than other studies, which shows that the accuracy of cytology is largely dependent on the level of expertise of the cytologist.[47 48] Although there are limitations in accuracy and quality assurance, the widespread application of cytology for screening in populations has successfully reduced the burden of cervical cancer, especially squamous cell cervical cancer, since the middle of the last century.[37] The implementation of HPV testing in all healthcare setting worldwide will take a long time, if possible at all, due to the strict laboratory conditions and large investment required. Therefore, in order to achieve the goal of worldwide elimination

of cervical cancer in a timely manner, primary cytology followed by triage by HPV testing is recommended for areas with limited resource where cervical cancer screening otherwise would be infeasible.[49] However, level of cytologist is the most important factor affecting the accuracy and cost-effectiveness in the general population, therefore special attention should be paid to train cytologists.

Several studies have shown that co-testing increases the screening cost compared with primary HPV.[25–27] And, co-testing is most suitable for well-developed areas with high levels of cytologists, but it is difficult to carry out in most areas of China and other LMICs.[15 34] Primary HPV requires HPV(−) individuals to be recalled every 5 years, while co-testing requires those who are HPV(−) with cytology ASC-US to be recalled every 3 years. Currently, it is unclear whether this shorter screening interval is justified, especially considering its increased costs and resource uses. There is no prospective cohort study on this topic, and our study is expected to provide data needed with follow-up in the future.

Our results supported that primary HPV testing was the preferred screening strategy among women aged 30–65 years, which was consistent with the evidence from the American Society for Colposcopy and Cervical Pathology.[6] A decision analysis showed that cytology alone resulted in the lowest benefit about life-years gained and number of CIN2 or CIN3 cases detected, and it has lower sensitivity for precancer than primary HPV testing or co-testing.[38] On the other hand, a retrospective study concluded that the combination of cytology and HPV testing (co-testing) offered very little incremental benefit in detection but increased the number of procedures and the risk for harms.[47]

This screening cohort has low loss to follow-up rate (11.9%), which increases the stability of our results. In addition, a rigorous diagnostic procedure performed by cytologists and pathologists (a diagnostic panel of three senior doctors) ensure the accuracy of the end point. However, our study also have limitations; first, the single-centre study limits the extrapolation of our results. Second, three strategies based on one population might have cross-contamination, and rigorous randomised controlled trial is preferred. Finally, although there is a strict quality control process for cytology and pathology results, HPV testing is based on only one technique of kit and may be biased.

In conclusion, guidelines from Europe or the USA recommend cervical cancer screening strategies in the general population. However, there is still a lack of real-world data on cervical cancer screening in China, and our study provides real-world data on cervical cancer screening. Our results show that primary HPV screening strategy should be adapted whenever resources permit. In resource-poor areas, primary cytology is an acceptable alternative. However, cytologists should be trained adequately to ensure acceptable cytology accuracy. Co-testing is costly, requires well-trained cytologists and it does not show improvement in clinical performance over primary HPV.

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