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ORIGINAL RESEARCH

Performance of the Human Papillomavirus E6/E7 mRNA Assay in the Primary Screening of Cervical Cancer: Opportunistic Screening in Fujian, China

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Purpose: A high-risk human papillomavirus E6/E7 mRNA (HR-HPV mRNA) assay is widely used in cervical cancer screening in China. However, it is still unclear whether stand-alone HR-HPV mRNA testing is sufficient for primary screening. The purpose of this study was to investigate the feasibility of a stand-alone HR-HPV mRNA assay for primary screening of cervical cancer.

Methods: Women aged 21 and older were recruited in Fujian Province, China, from January 2020 to January 2022. Cervical exfoliated cells were collected for cervical cytology and HR-HPV mRNA assays, and women with positive results on either assay were referred for colposcopy. The screening effectiveness of the assay was calculated based on the cervical histology. When comparing the efficacy of the different screening strategies, only women aged 25 and older were included.

Results: A total of 9927 women were recruited. This study identified 217 cases of high-grade squamous intraepithelial disease or worse (HSIL+). The overall age-specific HR-HPV infection rate showed a U-shaped distribution. The sensitivity of the HR-HPV mRNA assay to identify CIN2+ and CIN3+ was 97.2% and 97.9%, respectively, which was significantly higher than that of cytology (82.9% and 88.6%, P<0.001 and 0.002). The sensitivity of the HR-HPV mRNA primary screening strategy to identify CIN2+ and CIN3+ was 92.2% and 94.3%, respectively, which was similar to the co-testing strategy (P=0.336 and 0.394) and higher than the cytology primary screening (P=0.002 and 0.048). In addition, the HR-HPV primary screening strategy had a lower referral rate for colposcopy than cytology primary screening (5.4% vs 6.6%, P<0.001), and the screening cost was lower than co-testing ($29,594.3 per 1000 screened women vs $55,140 per 1000 screened women, P<0.001).

Conclusion: In conclusion, the detection of CIN2+/CIN3+ by HR-HPV mRNA is both specific and sensitive. It may be suitable for primary screening of cervical cancer in China.

Keywords: cervical cancer, HR-HPV, E6/E7 mRNA, primary screening, cervical intraepithelial neoplasia

Introduction

Cervical cancer is the fourth most common malignant tumor in women, and its incidence and mortality have been on the rise in China in recent years, with a trend of increasing impact on younger women.[1–3] The incidence of cervical cancer in China and Fujian Province was 10.7/100,000 and 21.0/100,000, respectively, and the mortality rate was 4.4/100,000.[4,5] Persistent infection with high-risk human papillomavirus (HPV) is a major causative factor for cervical cancer. Tertiary prevention of cervical cancer has been widely practiced worldwide.[2] The prevalence of cervical HR-HPV varies among women of different ages. Previous evidence has found that in developing countries, women under 25 years of age and women over 50 years of age exhibit high rates of HR-HPV infection, whereas women between 25 and 50 years of age have lower rates.[5–8]

HPV vaccination is the most effective way to prevent cervical cancer, but China's HPV vaccine was only first licensed in 2016,[9] and the coverage rate is low. Therefore, it is very important to improve our current screening system.

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Pap smears have performed unsatisfactorily in developing countries and regions, with a sensitivity of only 30%-40%.[10] The efficacy of fluid-based cytology has improved, but the number of cytopathologists in China remains inadequate, and the diagnostic techniques are immature, which leads to a hindrance in the popularization of this technique for routine screening. Screening methods using visual inspection with acetic acid and Lugol's iodine (VIA/VILI) are not dependent on specific equipment and are simple and inexpensive to perform, but their sensitivity is low (40–60%).[11,12]

Given HPV's major etiological role, HPV testing can be an accurate means of detecting women at risk for cervical cancer. In 2008, a high-risk HPV (HR-HPV) test was recommended in Europe for primary screening of cervical cancer in women over 25 years of age; in April 2014, the US Food and Drug Administration (FDA) approved the Cobas 4800 HPV-DNA test for primary cervical cancer screening of women over 25 years of age.[13] The latest WHO guidelines published in 2021 similarly recommend HPV testing for primary screening (WHO, 2021). HPV testing is relatively accurate and consistent regardless of the test used, with HPV primary screening increasing the detection of cervical intraepithelial neoplasia (CIN) at grade 2 or worse (CIN2+) by 25%.[14] The advantage of HPV primary screening is its high sensitivity, but it may lack specificity.

The four currently FDA-approved HPV tests include three DNA-based tests and one RNA-based test. The detection of HR-HPV E6/E7 mRNA has theoretically higher specificity.[15] It is well known that E6/E7 oncogenes play a key role in the development of cervical cancer. Since E6/E7 overexpression occurs after the integration of HPV into the genome, it is important in the detection of high-grade cervical lesions. Previous evidence[16,17] found that HR-HPV E6/E7 mRNA detection for cervical cancer screening can reduce the number of colposcopy referrals and reduce the number of HPV or cytology reviews, and thus reduce the cost of cervical cancer screening. Previous studies[18,19] have compared the efficacy of HR-HPV E6/E7 mRNA testing with that of HC2 and cytology testing in cervical cancer screening, which also confirmed that the HR-HPV E6/E7 mRNA test was valid and feasible. However, that study was limited by its small sample size. Therefore, there is a need for a large prospective screening study to assess the performance of the HR-HPV E6/E7 mRNA assay in cervical cancer screening in China. Direct detection of HR-HPV E6/E7 in cervical samples may be more specific than the HR-HPV-DNA assay.[20] The HR-HPV E6/E7 mRNA test met the cross-sectional clinical and reproducibility criteria for detecting CIN2+ in the International Guidelines for HR-HPV Testing Requirements in Cervical Cancer Screening in a noninferiority comparison with HR-HPV DNA testing.[21]

Therefore, through a large cohort study of cervical cancer screening in Fujian Province, China, this study evaluated the efficacy of different primary screening programs and various triage strategies to improve the level of cervical cancer prevention in China.

Materials and Methods

Study Population

Women who underwent cervical cancer screening at Fujian Maternal and Child Health Hospital from January 2020 to January 2022 were recruited for this study. The inclusion criteria were as follows: age ≥21 years; no serious organ dysfunction or mental illness; voluntary participation; and able to complete the questionnaire. The exclusion criteria were as follows: women with a history of hysterectomy; previous diagnosis of CIN or cervical cancer; pelvic radiation therapy; women who were pregnant or breastfeeding; and women being treated for other serious medical and surgical diseases. This study was approved by the Ethics Committee of Fujian Maternal and Child Health Hospital (approval number: 2020YJ239).

Study Design

One cervical specimen was collected from all participants at enrollment using a cell brush and preserved in suspension in PreservCyt collection media (Hologic Inc., MA, USA). Each specimen was used for liquid-based cytology (TCT) assays and Aptima HR-HPV mRNA assays (Hologic, CA, USA).

Participants with a cytologic diagnosis of atypical squamous cells of undetermined significance (ASCUS) or worse and/or positive on the HR-HPV mRNA assay were referred for colposcopy. Colposcopy was performed by a senior colposcopy physician with at least 10 years of experience. If abnormal epithelial cells were observed colposcopically,

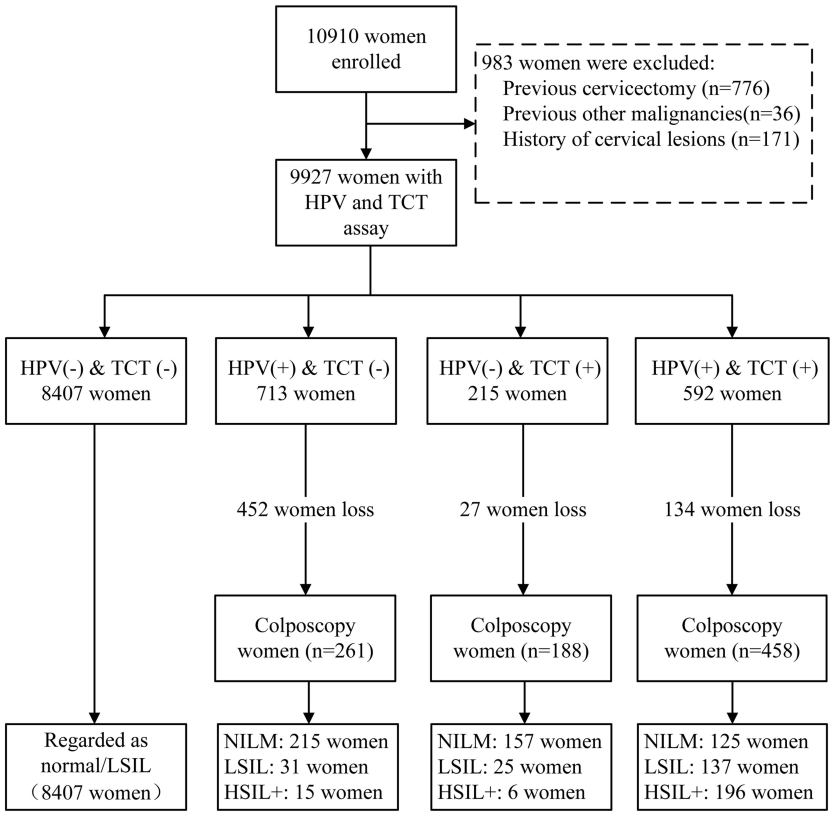

Figure 1 Flowchart of the study.
Abbreviations: HPV(-), high-risk human papillomavirus mRNA assay was negative; HPV(+), high-risk human papillomavirus mRNA assay was positive; TCT(-), Cervical cytology result was normal; TCT(+), Cervical cytology result was abnormal; NILM, negative for intraepithelial lesion or malignancy; LSIL, low grade squamous intraepithelial lesion; HSIL+, high grade squamous intraepithelial lesion or worse.

a colposcopy-guided biopsy was performed. If the colposcopic evaluation was inadequate, random biopsies were performed at the 3, 6, 9 and 12 o'clock positions of the cervix, and endocervical curettage (ECC) was performed.

Patients with ASCUS or low-grade squamous intraepithelial lesions (LSIL) on cytology and no obvious lesions on colposcopy at the first visit were not biopsied and were considered to have a histological status of "no high-grade squamous intraepithelial disease (HSIL)". If cervical cancer is suspected at the time of sampling, a cervical biopsy is performed immediately.

Women with a negative combined screening result were considered to have a histological status of "normal/LSIL". Biopsy results were classified into the following three broad groups: negative for intraepithelial lesion or malignancy (NILM, including no pathologic changes and benign or reactive lesions), LSIL (including CIN 1, HPV-affected), and high-grade cervical lesions or worse (HSIL+, including CIN2, CIN3 and cancer) (Figure 1).

The Process of the Different Referral Strategies

Three triage strategies were analyzed in this study: (1) Co-testing primary: Patients with both HR-HPV mRNA positive and abnormal cytology (ASCUS or worse) results were referred to colposcopy directly; patients with positive HPV-16/-18/-45 mRNA were directly referred for colposcopy, regardless of the cytology results (Figure 2A). (2) Primary cytology: Patients with cytology results of ASCUS were referred for colposcopy if they tested positive for HR-HPV mRNA. Patients with cytology results of LSIL or worse were referred to colposcopy directly regardless of the HR-HPV mRNA results (Figure 2B). (3) HR-HPV mRNA primary: For HR-HPV-positive patients, further genotyping should be performed. When HPV-16/-18/-45 is positive, they will be directly referred for colposcopy testing, or other genotype-positive patients with a cytology test result of ≥ASCUS will be referred for colposcopy testing (Figure 2C).

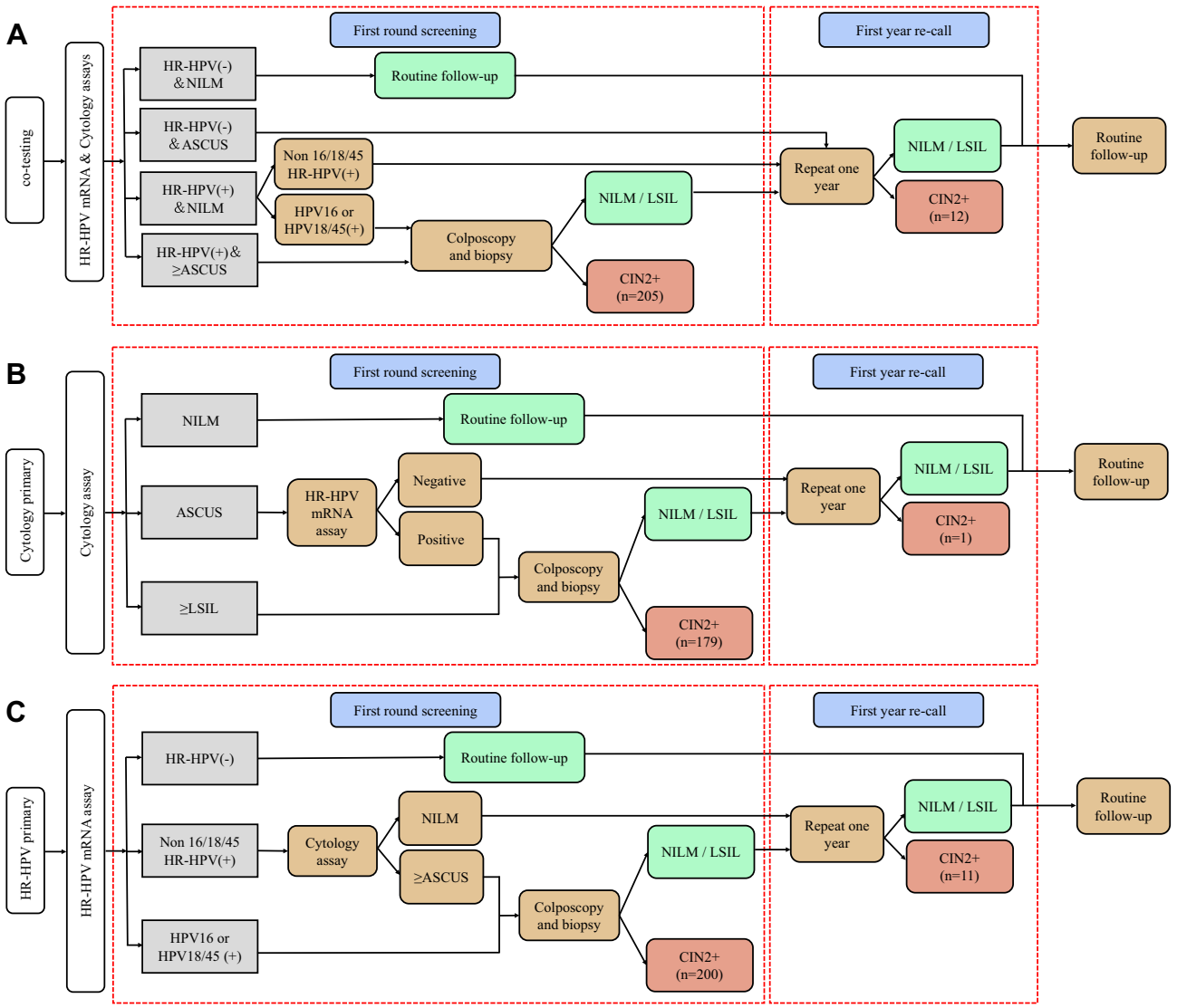

Figure 2 Processes for different cervical cancer screening referral strategies.

Notes: (**A**) Co-testing primary: Patients with both HR-HPV mRNA positive and abnormal cytology (ASCUS or worse) results were referred to colposcopy directly; patients with positive HPV-16/-18/-45 mRNA were directly referred for colposcopy, regardless of their cytology results. (**B**) Primary cytology: Patients with cytology results of ASCUS were referred for colposcopy if they tested positive for HR-HPV mRNA. Patients with cytology results of LSIL or worse were referred to colposcopy directly. (**C**) HR-HPV mRNA primary: For HR-HPV-positive patients, further genotyping should be performed. When HPV-16/-18/-45 is positive, they will be directly referred for colposcopy testing, or other genotype-positive patients with a cytology test result ≥ASCUS will be referred for colposcopy testing.

Abbreviations: HR-HPV(-), high-risk human papillomavirus mRNA assay was negative; HR-HPV(+), high-risk human papillomavirus mRNA assay was positive; ASCUS, atypical squamous cells of undetermined significance; ≥LSIL, low grade squamous intraepithelial lesion or worse; CIN2+, cervical intraepithelial neoplasia grade 2 or worse.

According to the ASCCP guidelines,[22] HPV primary screening strategies are recommended for women aged 25 years and older. When evaluating the efficacy of the 3 different referral strategies, we excluded women 21–24 years of age and only analyzed women 25 years of age and older. For women diagnosed with CIN2+, according to the 2019 version of the ASCCP guidelines,[23] women with CIN2 younger than 25 years old can be followed up for 12 months, and those aged 25–30 years who are concerned about the impact of cervical resection on subsequent pregnancy can be followed up for 12 months, but cervical excision should be performed if disease progression occurs. Cervical/hysterectomy was performed on CIN2 women ≥30 years of age and all CIN3+ women.

Liquid-Based Cytology (TCT) Assay

All samples were first subjected to TCT testing. The TCT results were assessed according to the 2014 Bethesda system by expert pathologists. For TCT detection, the cervical exfoliated cell suspension is first made into a single layer of cells on a glass slide and then fixed and Pap stained. The pathologist reads the slide under a microscope. The results are classified as follows: negative for intraepithelial lesion or malignancy (NILM); atypical squamous cells of undetermined significance (ASC-US); low-grade squamous intraepithelial lesion (LSIL); atypical squamous cells, not possible to exclude high-grade squamous intraepithelial lesion (ASC-H); high-grade squamous intraepithelial lesion (HSIL); squamous cervical cancer; atypical glandular cells (AGCs); and adenocarcinoma in situ (AIS).

HR-HPV mRNA Assay and Genotyping

TCT specimens were tested under blinded conditions using the Aptima® HR-HPV assay (Gen Probe; Hologic, CA, USA), an FDA-approved HR-HPV E6/E7 mRNA assay that detects 14 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). All HR-HPV-positive samples were further genotyped for HPV-16, HPV-18, and HPV-45 using the Aptima® HPV 16 18/45 genotype test. Testing and reporting of the results was performed by a professional technician following the manufacturer's instructions.

Data Analysis

Histologically confirmed HSIL+ was used as the endpoint for clinical observation. Sensitivity and specificity for the detection of HSIL+ were determined according to standard definitions. The 95% confidence intervals (CIs) were calculated according to the binomial method. The De Long test was used to determine statistical significance ($P<0.05$). The number of referrals for colposcopy performed to detect a single case of HSIL+ was calculated and used as a measure of the efficiency of referral for the screening method. There were two definitions of cost used: the first was the cost per 1000 women screened in the first round of screening, and the second was the cost per HSIL+ (CIN2+) case identified in the first round of screening. Age was expressed as the median and interquartile range because it was not normally distributed. Differences between categorical variables were compared using the chi-squared ($\chi 2$) test. Statistical analysis was performed using SPSS 24.0 (IBM, New York, NY, USA), and $P<0.05$ was considered statistically significant.

Results

Characteristics of the Study Population

A total of 10,910 women were recruited for this study from January 2020 to January 2022. Among these, 983 women were excluded because they met the exclusion criteria, and 9927 eligible participants were included in the analysis. They provided a sample of exfoliated cervical cells and a completed questionnaire. The median age of the participants was 43 years (interquartile range: 36–50). Of the 9927 women with both cytology and HR-HPV mRNA screening results, 8.1% (807/9927) had a positive cytology result (ASCUS or worse), and 13.1% (1305/9927) were infected with HR-HPV mRNA. Of these, 13.5% (176/1305) were HPV16 mRNA positive, and 7.4% (97/1305) were HPV18/45 positive. Among women with a cytological diagnosis of ASCUS or worse, HR-HPV mRNA positivity was 73.4% (592/807). Of the 1520 women who tested positive for either screening test, 907 (59.7%) underwent colposcopy (Figure 1).

The prevalence of HR-HPV mRNA infection was analyzed in ten different age subgroups (Figure 3). The highest prevalence of HR-HPV mRNA was found in the 21–25 years age group (22.7%), followed by the 61–65 years age group (22.3%). The lowest prevalence was in the 41–45 years age group (11.2%). The overall age-specific HR-HPV mRNA prevalence showed a U-shaped distribution. The age-specific HPV16 mRNA prevalence was similarly U-shaped, but the HPV18/45 mRNA prevalence was highest in women >65 years old (2.8%) and lowest in women 21–25 years old (0.8%).

Detection Rate of HSIL and the Above Lesions

In this study, 613 women indicted for the procedures by the study protocol did not undergo colposcopy and biopsy because of loss to follow-up. In addition, among those who were judged to have inadequate colposcopy due to unclear exposure of the

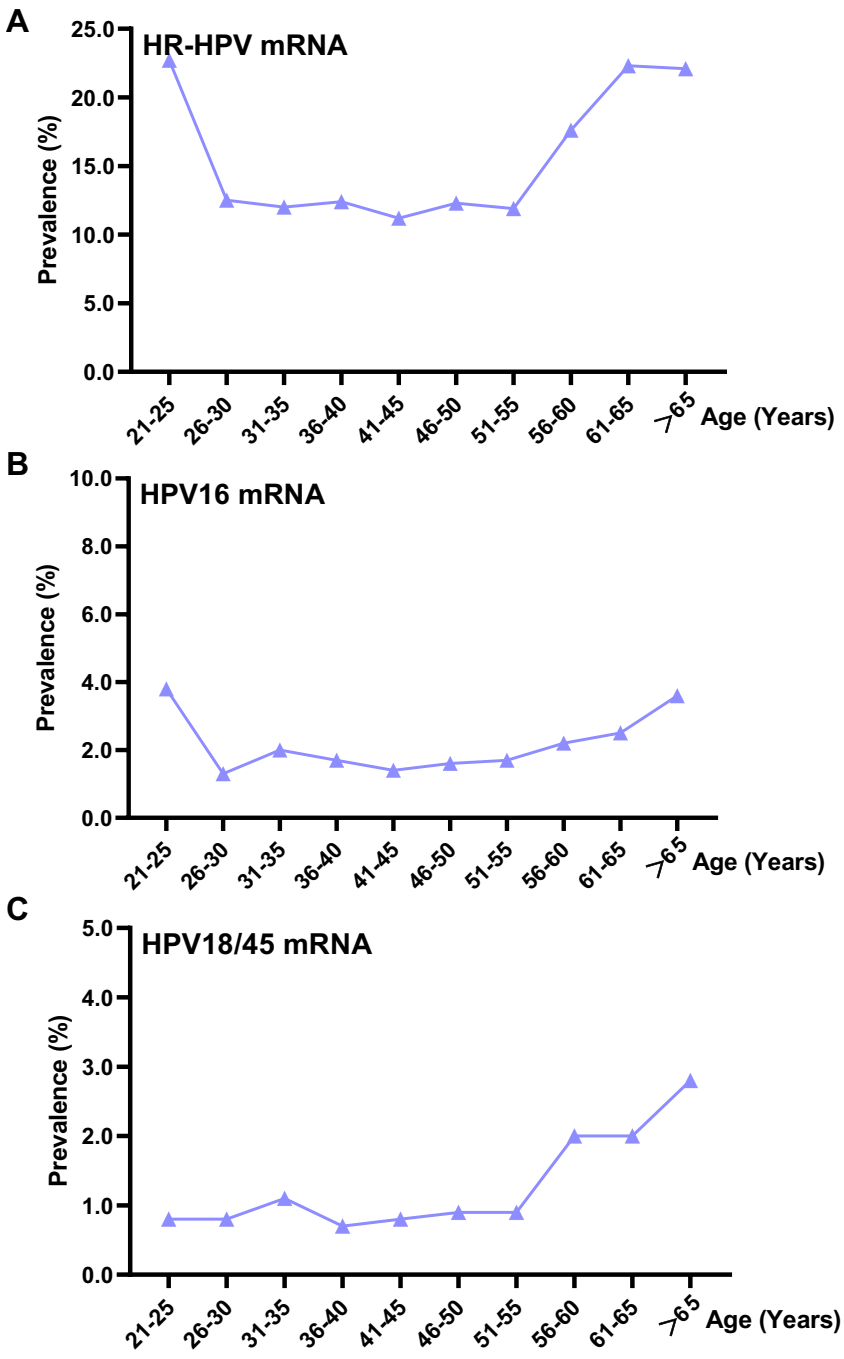

Figure 3 High-risk human papillomavirus mRNA prevalence in women of different ages.
Notes: (**A**) line chart of positive rates of HR-HPV mRNA in women of different ages; (**B**) line chart of positive rates of HPV16 mRNA in women of different ages; (**C**) line chart of positive rates of HPV18/45 mRNA in women of different ages.
Abbreviations: HR-HPV, high-risk human papillomavirus; HPV18/45 mRNA, any mRNA positive for HPV18 and HPV45.

cervical squamous-columnar junction, 86 women underwent additional endocervical scraping (ECC) to determine the presence of cervical lesions. Ultimately, 9314 women had histopathological results for the follow-up analysis.

A total of 217 (2.3%) women were identified as CIN2+. The HR-HPV infection rate of cervical CIN2+ women was significantly higher than that of normal/LSIL women (97.2% vs 5.6%, P<0.001). The age of first sex, previous pregnancy, weekly alcohol use and smoking did not differ between CIN2+ and normal/LSIL women (P=0.202, 0.236, 0.735, 0.527, respectively, Table 1).

Table 1 Characteristics of the Participants in This Study. (N=9314)

Variables	Normal/LSIL (n=9097)	HSIL+ (CIN2+) (n=217)	*P* value
Age, years (mean±SD)	43.1±10.2	46.1±13.1	0.001
Age of first sex, years (mean±SD)	21.3±2.8	20.5±3.1	0.202
Previous pregnancy, n/N (%)			0.236
Yes	92.3% (8397/9097)	94.5% (205/217)	
No	7.7% (700/9097)	5.5% (12/217)	
Weekly alcohol use, n/N (%)			0.735
Yes	11.7% (1064/9097)	12.4% (27/217)	
No	88.3% (8033/9097)	87.6% (190/217)	
Smoking, % (n/N)			0.527
Current smoker	6.6% (601/9097)	5.5% (12/217)	
Non-smoker	93.4% (8496/9097)	94.5% (205/217)	
HR-HPV mRNA			<0.001
Negative	94.4% (8589/9097)	2.8% (6/217)	
Positive	5.6% (508/9097)	97.2% (211/217)	

Abbreviations: LSIL, low grade squamous intraepithelial lesion; HSIL+, high grade of squamous intraepithelial lesion or worse; CIN2+, cervical intraepithelial neoplasia grade 2 or worse.

Comparison of the Efficacy of Different Screening Methods

Positive screening by cytology was defined as ASCUS or worse (ASCUS+). Positive screening for HR-HPV mRNA was defined as positive for any type of HR-HPV mRNA infection. The comparative efficacy of the two screening methods in detecting HSIL+ (CIN2+/CIN3+) is shown in Table 2. The sensitivity of the HR-HPV mRNA assay to detect CIN2+ and CIN3+ was 97.2% and 97.9%, respectively, which was significantly higher than that of cytology at 82.9% (P<0.001) and 88.6% (P=0.002). The specificity of the HR-HPV mRNA assay to detect CIN2+ and CIN3+ was 94.4% and 93.7%, respectively, slightly lower than that of cytology at 95.1% and 94.5%.

Comparison of the Efficacy of the Different Referral Strategies

HR-HPV mRNA primary and co-testing primary screening strategies identified 200 and 205 CIN2+ women in the initial screening, higher than the number for cytology primary screening (179 women). HR-HPV mRNA primary and co-testing primary screening had the highest sensitivity for identifying CIN2+/CIN3+, significantly higher than cytology primary screening (CIN2+: $P_{HPV\text{-}Cy}$=0.002, $P_{Co\text{-}Cy}$<0.0001; CIN3+: $P_{HPV\text{-}Cy}$=0.048, $P_{Co\text{-}Cy}$=0.013). However, the specificity of the three screening strategies to identify CIN2+/CIN3+ was similar (CIN2+: $P_{HPV\text{-}Cy}$=0.803, $P_{Co\text{-}Cy=}$0.114; CIN3+: $P_{HPV\text{-}}$

Table 2 Comparison of Performance of Different Screening Assays. (N=9145)

Screening Assays	Cytology	HR-HPV mRNA	*P* value
CIN2+			
Sensitivity (95% CI)	82.9 (77.8, 87.9)	97.2 (95.0, 99.4)	<0.001
Specificity (95% CI)	95.1 (94.7, 95.6)	94.4(93.9, 94.9)	0.033
NPV (95% CI)	99.6 (99.1, 99.9)	99.9 (99.8–100.0)	<0.001
PPV (95% CI)	28.7 (24.1,32.6)	29.2 (25.1–33.6)	0.834
CIN3+			
Sensitivity (95% CI)	88.6 (83.3, 93.8)	97.9 (95.5, 100.0)	0.002
Specificity (95% CI)	94.5 (94.1, 95.0)	93.7 (93.2, 94.2)	0.010
NPV (95% CI)	99.8 (96.7, 99.9)	99.9 (96.1, 100.0)	0.003
PPV (95% CI)	19.9 (12.5, 28.5)	19.1 (12.9, 29.1)	0.706

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; NPV, negative predictive value; PPV, positive predictive value.

Table 3 Comparison of Performance of Different Triage Strategies. (N=9145)

Triage Strategies	Cytology Primary	Co-Testing Primary	HR-HPV mRNA Primary	*P* value[a]	*P* value[b]	*P* value[c]
CIN2+						
Sensitivity (95% CI)	82.5 (77.4, 87.5)	94.5 (91.4, 97.5)	92.2 (88.6, 95.7)	<0.001	0.002	0.336
Specificity (95% CI)	96.8 (96.4, 97.1)	96.3 (95.9, 96.7)	96.7 (96.3, 97.1)	0.114	0.803	0.183
NPV (95% CI)	99.6 (99.2, 99.9)	99.9 (99.3, 100.0)	99.8 (99.2, 100.0)	<0.001	0.005	0.359
PPV (95% CI)	37.8 (34.9, 39.1)	38.0 (36.5, 41.5)	39.9 (36.9, 43.1)	0.930	0.490	0.533
No. of case at initial screening	179	205	200	/	/	/
No. of case at 1-year repeat	1	12	11	/	/	/
CIN3+						
Sensitivity (95% CI)	88.6 (83.3, 93.8)	96.4 (93.4, 99.5)	94.3 (90.4, 98.1)	0.013	0.048	0.394
Specificity (95% CI)	96.2 (95.8, 96.6)	95.6 (95.2, 96.0)	96.0 (95.6, 96.4)	0.045	0.470	0.198
NPV (95% CI)	99.8 (99.7, 99.9)	99.9 (99.5, 100.0)	99.9 (99.7, 100.0)	0.017	0.104	0.410
PPV (95% CI)	26.2 (23.5, 28.5)	25.0 (22.3, 27.6)	26.3 (23.5, 28.5)	0.685	0.947	0.631
No. of case at initial screening	124	135	132	/	/	/
No. of case at 1-year repeat	0	5	5	/	/	/
Referred to colposcopy (%)	6.6% (614/9314)	5.8% (539/9314)	5.4% (501/9314)	0.023	<0.001	0.225
Number of colposcopy referrals per case of HSIL+ identified	3.4 (614/179)	2.6 (539/205)	2.5 (501/200)	0.024	0.008	0.679
Total cost per 1000 screened women at first round screening[d]	¥228,400 ($32,628.6)	¥385,980 ($55,140)	¥207,160 ($29,594.3)	<0.001	0.029	<0.001
Cost per identified CIN2+ women at first round screening	¥1268.9 ($181.3)	¥1778.7 ($254.1)	¥981.8 ($140.3)	0.001	0.011	<0.001

Notes: *P* value[a], comparison of cytology and co-testing; *P* value[b], comparison of cytology and HR-HPV mRNA assay; *P* value[c], comparison of co-testing and HR-HPV mRNA assay; [d]Cost refers to all medical direct costs consumed by the screened woman in the hospital; the price of the HR-HPV mRNA assay is RMB ¥140 (US$ 20.0) per person per time, and the price of cytology is RMB ¥180 (US$ 25.7) per person per time, the price of colposcopy is RMB ¥550 (US$ 78.6) per person per time. The equivalent value between the RMB ¥ and the USD was calculated at 7:1.

Abbreviations: CIN2+/3+, cervical intraepithelial neoplasia grade 2/3 or worse; NPV, negative predictive value; PPV, positive predictive value.

$_{Cy}$=0.470, $P_{Co\text{-}Cy}$=0.045). This study found that the HR-HPV mRNA screening strategy had the lowest colposcopy referral rate (5.4%), with only 2.5 colposcopy visits per identified CIN2+ woman. For the analysis of screening cost, it was found that the medical cost consumption of the HR-HPV mRNA strategy in one round of screening was significantly lower than that of the cytology screening (P=0.029) and co-testing (P<0.001) strategies (Table 3).

Discussion

This study evaluated the efficacy of HR-HPV E6/E7 mRNA and liquid-based cytology assays for screening and three different triage strategies in a hospital-based opportunistic screening cohort of 10,910 women in China. We found that the HR-HPV mRNA assay has the highest sensitivity and good specificity in detecting CIN2+/CIN3+ lesions.

Previously, large population-based studies in China reported HR-HPV infection rates ranging from 9.9% to 27.5%.[24] In this study, the HR-HPV mRNA positive rate was 13.1%, which is at the low end of the range of reported HPV infection rates in China. There may be two reasons for the low infection rate. First, this study tested for E6/E7 mRNA of HR-HPV, and previous studies have found that the positive rate of HR-HPV E6/E7 mRNA is lower than that of HR-HPV DNA.[25] Second, Fujian Province may not be a high prevalence population for HR-HPV in the Chinese region.

The International Agency for Research on Cancer (IARC) study reported a U-shaped distribution of HR-HPV prevalence in developing countries. Specifically, the prevalence of HR-HPV peaks among women under 25 years of age and then slowly declines to a trough at 45–55 years of age. It then slowly rises, reaching a second peak among women over 55 years of age.[7,26] Similarly, a large population-based study conducted in 37 cities in China in 2015 reported a "bimodal" pattern of HR-HPV prevalence, with peaks occurring at ages 15–19 and 50–60 years.[27]

In the present study, a similar U-shaped pattern of HR-HPV mRNA infection was observed, although the largest, second peak occurred at ages >60 years. This finding can be explained by the fact that postmenopausal women are at high risk for HPV infection. Possible reasons for this include the following: postmenopausal women and their sexual partners may have altered patterns of sexual behavior; atrophy of the cervical and vaginal mucosa may make these women more vulnerable to microdamage, immunosuppression, and persistent viral infection; and they may not be vaccinated. Therefore, postmenopausal women are a critical population to target for the prevention and treatment of cervical cancer.

The advantages of HR-HPV testing are that it is objective, the results are available in a short period of time, and it is easily reproducible. HPV testing can detect precancerous cervical lesions much earlier than cytology. Negative results of HR-HPV testing have been reported to predict a low risk of future CIN2+ and may allow such individuals to reduce their frequency of screening.[14,28,29] Theoretically, HR-HP mRNA is only produced after integration of the HPV viral genome and it may represent an active HR-HPV infection, so detection of HR-HPV mRNA transcripts may provide greater specificity for CIN2+.[30]

HR-HPV mRNA testing has received attention in recent years, with several studies comparing its performance in cervical cancer screening to HPV DNA testing. For example, one study[15] enrolled 9451 women aged 30–60 years attending routine cervical cancer screening in Germany and compared an RNA-based test with a DNA-based test and found no statistically significant difference in the sensitivity of the two in detecting CIN2+ or CIN3+ lesions. However, the HR-HPV mRNA test had a higher specificity and positive predictive value than the HR-HPV DNA assay. HR-HPV RNA-based assays only detect actively infected cells, whereas DNA-based assays cannot distinguish between intracellular and extracellular viral DNA, leading to the possibility that the results are influenced by contamination with extracellular viral particles. In line with this, in other earlier studies, RNA-based HR-HPV assays were slightly less sensitive in detecting CIN2+.[31] In addition, more recent reports have shown equal[32,33] or higher sensitivity for HR-HPV mRNA testing compared to DNA testing.[18,34] After long-term follow-up, women who tested negative for HR-HPV mRNA were found to have a fairly low risk of future HSIL+, as were women who tested negative for DNA-based tests.[35,36] In this study, the HR-HPV mRNA assay showed good performance in opportunity-based cervical cancer screening with a high sensitivity of 97.2% and a high specificity of 94.4%. Therefore, the HR-HPV mRNA assay could be suitable for primary cervical cancer screening.

The current strategy in China is mainly to perform annual cytological screening. The reported detection rate of cervical precancerous lesions and cervical cancer by cytology screening is only 11.57/100,000 in China.[37,38] CIN2+ was found in 217 (2.3%) of the women included in this study, among whom the HR-HPV mRNA assay identified 211 women with CIN2+ compared to 180 women with the cytology assay. The HR-HPV mRNA assay had higher sensitivity in identifying CIN2+/CIN3+ than the cytology assay ($P<0.001$, $P=0.002$). Therefore, the poor sensitivity of cytology requires the development of more accurate screening methods. Previous studies found HR-HPV testing to be a more sensitive screening method than cytology, and HR-HPV mRNA assays to further improve the detection of CIN2+ cases have been developed and evaluated.[31–34]

In this study, the CIN2+ detection rate for co-testing screening strategy was 2.2%. The detection rate of CIN2+ by the HR-HPV mRNA primary screening strategy was 2.1%, but the detection rate by the cytology primary screening strategy was only 1.9%. The sensitivity and specificity of the HR-HPV primary screening strategy for CIN2+/CIN3+ identification were similar to those of the co-testing screening strategy (sensitivity: $P=0.336$, $P=0.394$; specificity: $P=0.183$, $P=0.198$), but the sensitivity was significantly higher than that of the cytology primary screening strategy. Given that HR-HPV mRNA primary screening has a lower referral rate for colposcopy ($P<0.001$) and a lower screening cost ($P=0.029$) than cytology primary screening strategies, it is more often recommended for cervical cancer screening in China. Because of the higher cost of co-testing screening, it may only be applicable to economically developed areas of China. Cost is an important factor to consider when developing a screening strategy, especially in low- and middle-income countries where resources are relatively scarce.[39] Even though the HR-HPV mRNA primary screening strategy is low cost, the number of CIN2+ cases it identifies is equal to or higher than other, more expensive screening strategies. This may be a more cost-effective cervical cancer screening strategy for China, which has low screening coverage and a large population.

Although the sensitivity of primary cytological screening strategies in women over 25 years of age is low, primary cytological screening strategies are still recommended for cervical cancer screening in women under 25 years of age. This is because women aged 21–24 have a high prevalence of HPV infection.[5] This study also found a high prevalence of HPV mRNA in women aged 21–24, but women in this age group have a low incidence of cervical lesions.[40] Therefore, ASCCP guidelines still recommend cytological screening as a recommended strategy for women under 25 years of age.[23]

This study has some limitations that need to be considered. First, when analyzing the age-specific HR-HPV prevalence, few women older than 65 were analyzed. Second, 40.3% of women who were indicated for referral for colposcopy did not attend it. Third, longitudinal follow-up should be performed in women with negative results for both cytology and HR-HPV mRNA.

Conclusions

In summary, the HR-HPV mRNA assay is more sensitive than cytology for cervical cancer screening, with good specificity. The results of this study suggest that an HR-HPV mRNA primary screening strategy is a suitable alternative for cervical cancer screening in China.

Data Sharing Statement

The data used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

We confirm that our study complies with the Declaration of Helsinki. The study protocol was approved by the ethics committee of Fujian Maternal and Child Health Hospital (approval number: 2020YJ239). Informed consent in our study was waived because of its retrospective nature and anonymous analysis.

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Disclosure

The authors declare that they have no competing interests.

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