

The Triage Effectiveness of an Extended High-Risk Human Papillomavirus Genotyping Assay for Women with Cytology Showing Atypical Squamous Cells of Undetermined Significance in China

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Diling Pan ^{1,*}
Binhua Dong ^{2,3,*}
Hangjing Gao ²
Xiaodan Mao ^{2,3}
Huifeng Xue⁴
Pengming Sun ^{2,3}

¹Department of Pathology, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou 350001, Fujian, People's Republic of China; ²Department of Gynecology, Laboratory of Gynecologic Oncology, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian 350001, People's Republic of China; ³Fujian Key Laboratory of Women and Children's Critical Diseases Research, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian 350001, People's Republic of China; ⁴Fujian Provincial Cervical Disease Diagnosis and Treatment Health Center, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou 350001, Fujian, People's Republic of China

*These authors contributed equally to this work

Purpose: Little is known about the performance of extended high-risk human papillomavirus (HR-HPV) genotyping triage of cytology showing atypical squamous cells of undetermined significance (ASC-US). This study aims to evaluate the effectiveness of triage with different HR-HPV genotype models among women with ASC-US.

Materials and Methods: In this study, all women who underwent cervical cytology and HR-HPV genotyping were enrolled from 2014 to 2017 in China, and those with cytology showing ASC-US were referred for colposcopy and/or biopsy. The endpoint was histological detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+). The outcome indicators were the sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs) and colposcopy referral rates.

Results: In all, 56,788 women were enrolled in this study, and 2658 (4.97%) women were reported to have ASC-US; 10.1% (242/2393) of women with ASC-US were identified as having CIN2+. The HR-HPV infection rate was 95.0% among all women with ASC-US who were identified as CIN2+, and the top five genotypes with prevalence and risk of CIN2+ were HPV16 (OR=26.38), HPV58 (OR=7.04), HPV18 (OR=4.44), HPV33 (OR=3.38), HPV31 (OR=2.97) and HPV52 (OR=2.96). The HPV16/18/31/33/52/58 model achieved higher sensitivity [91.3 (87.8–94.9)], specificity [70.0 (68.1–72.0)], PPV [25.5 (22.4–28.2)] and NPV [98.6 (97.3–98.7)] for the triage of ASC-US patients than the other HR-HPV-type combination models, but the colposcopy referral rate (36.2%) was significantly lower than that of the recommended HR-HPV nongenotyping model (47.6%).

Conclusion: This study confirms that the specific HR-HPV genotype HPV16/18/31/33/52/58 is an alternative strategy for ASC-US triage and can effectively reduce the high burden of colposcopy referrals in China.

Keywords: human papillomavirus, genotyping, atypical squamous cells of undetermined significance, cervical intraepithelial neoplasia

Introduction

Cervical cancer remains a high burden on global public health, with 530,000 new cases of cervical cancer diagnosed worldwide each year;¹ however, cervical cancer is relatively easy to prevent by screening.² Thus, it is particularly important to optimize screening programs to reduce the burden of cervical cancer. When atypical transformation is detected by cytology, further treatment and/or follow-up may be

Correspondence: Pengming Sun
Fujian Maternity and Child Health Hospital,
Affiliated Hospital of Fujian Medical
University, 18 Daoshan Road, Fuzhou
350001, Fujian, People's Republic of China
Tel +86-591-87558732
Fax +86-591-87551247
Email sunfemy@hotmail.com

needed based on the severity of the lesion.^{3,4} However, it remains a pending issue for the management strategy of women with atypical squamous cells of undetermined significance (ASC-US).⁵ ASC-US is not a specific diagnosis of progression or disease; rather, this indistinct diagnosis encompasses normal, low-grade squamous intraepithelial lesions (LSILs), high-squamous intraepithelial lesions (HSILs) and invasive cancer.⁶ In 2020, the American Society for Colposcopy and Cervical Pathology recommended immediate HPV testing, but 12-month repeat cytology was considered acceptable.⁷ In recent years, in many countries including the United States, immediate HPV testing is recommended as the preferred strategy after cytology showing ASC-US.⁸ Although most cases of ASC-US resolve with no apparent clinical disease, 15–20% are confirmed by histology to be cervical epithelial neoplasia (CIN) during follow-up.⁹ Previous research has indicated that the risk of CIN3 or worse among women with ASC-US is significantly higher than that among women without ASC-US.¹⁰ Therefore, an accurate category for these patients is needed to determine who needs subsequent management.

Research has confirmed that persistent infection with HR-HPV is a major factor in the development of cervical cancer.^{11,12} In recent years, HR-HPV testing has been recommended for the management of women with ASC-US, and colposcopy is recommended for all HR-HPV-positive cases.¹³ However, this management strategy may be overtreatment,¹³ because of the different carcinogenicities of the different HPV genotypes, the role of HPV in the development of CIN and cervical cancer varies with HPV genotype. The types of HPV infection related to cervical cancer are HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.¹⁴ Infection with HPV16 and HPV18 was found in 71% of women with invasive cervical cancer.¹⁵ A previous study¹⁶ indicated that the incidence of CIN2 or worse (CIN2+) at a follow-up of 13.4 years among HPV-infected women with initially normal cytology was as follows: 28.5% for those with HPV16 alone, 15.4% for those with HPV18 alone, 19.1% for those with HPV33, 18.2% for those with HPV35, 16.7% for those with HPV58, 15.7% for those with HPV31, 8.6% for those with HPV51, 8.5% for those with HPV45, 4.7% for those with HPV52, 3.6% for those with HPV39, and 2.8% for those with HPV56. These data provide a reference for triaging patients with ASC-US to select appropriate HPV genotypes.

The ideal triage strategy for patients with ASC-US is to maximize the sensitivity and specificity, thereby reducing the rate of misdiagnosis and unnecessary colposcopy.

Herein, our research was conducted to examine the performance of different HPV genotype combined models for women with ASC-US prior to the detection of CIN2+ and offers new parameters to design an optimal strategy for the triage of patients with ASC-US.

Materials and Methods

Study Subjects

A cervical cancer screening group of 56,788 women was set up from January 2014 to December 2017 in Fujian Province. All participants underwent cervical cytology and HR-HPV genotyping assessments. Women who met the following criteria were enrolled in the research: patients aged 21 years or older; those with a history of sexual activity; those with cytology and HR-HPV genotyping for cervical cancer screening; and those who signed informed consent. Women who met the following criteria were excluded: those with a history of hysterectomy, cervical surgery, or cervical cancer treatment; those who are currently pregnant; those with severe immunodeficiency disease; those who had undergone cytology and HR-HPV testing within three years; and those who refused to sign the informed consent. Among them, women with ASC-US confirmed by cytology who underwent colposcopy and/or biopsy were finally eligible for our study. The Ethics Committees of the Fujian Maternity and Child Health Hospital granted approval for this study (2014-045). All participants in this study provided written informed consent.

Screening Protocols

Prior to registration, informed consent was obtained from each included woman. Then, a confidential questionnaire-based interview was performed by an experienced doctor to evaluate each patient's medication and surgical history for cervical lesions; education level; history of smoking and drinking; and fertility history.

All qualified participants underwent gynecological examinations of the vulva, vagina and cervix. A doctor used a speculum to examine and collect cervical cells with plastic brushes. The cervical cells were collected in ThinPrep[®] Pap Test PreservCyt[®] Solution (Hologic Inc., Madison, WI, USA). A ThinPrep[®] Cytologic Test and HR-HPV genotyping test were performed on ThinPrep cervical cells. If the liquid-based cytology test (TCT) results showed ASC-US, colposcopy and biopsy/ECC were performed within 10 weeks. A completely visible cervical

transition area indicated satisfactory colposcopy; cervical biopsy was performed directly on visible lesions for women with abnormal colposcopy results; if colposcopy proved normal, a biopsy was not required. If colposcopy was unsatisfactory (the cervical transition area was not completely visible), endocervical curettage (ECC) was subsequently conducted. All enrolled participants were followed up for 12 months (Figure 1).

HR-HPV Genotyping Assay

The HR-HPV genotyping assay was performed using a PCR-reverse dot blot HPV genotyping kit (Yaneng Bioscience Co., Ltd., China) according to the instructions of manufacturer. The test can identify and distinguish 14 types of HR-HPV DNA in cervical exfoliated cells to detect each genotype based on nucleic acid amplification polymerase chain reaction (PCR) and point hybridization.

Cytology and Histology

The TCT and Bethesda System (TBS) were applied for cytology testing, and the CIN classification system was used for histology. For all histological results, the worst result was defined as the final result for each woman. If no biopsy was performed, or if the histological result was negative, the woman was regarded as CIN negative. Cell pathologists and histopathologists of Fujian Maternity and Child Health Hospital prepared and read the films. A session was convened to standardize the treatment protocols of colposcopy and histopathological diagnosis before the beginning of the study.

Statistical Analysis

Analyses were carried out with SPSS version 24.0 (IBM Corporation, New York, USA). The number of HR-HPV-positive patients with ASC-US divided by the total number of patients with ASC-US was regarded as the referral rate. The mean and standard deviation were calculated for the categorical variables. Values and percentages were also reported. We calculated the odds ratio (OR) of age and different HPV types to evaluate the risk of CIN2+ among women with ASC-US. We combined the HPV genotype in turn according to the OR to build the HR-HPV genotyping model. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the HR-HPV genotyping model for CIN2+ were evaluated and compared with the gold standard of pathological diagnosis. All of these results were used to assess the performance of

HR-HPV genotypes in the triage of women with ASC-US. The significance level was set at a P-value <0.05.

Results

Characteristics for Women with Cytology Showing ASC-US

Of the 56,788 women recruited, 53,464 were eligible and included in the study, 2658 (5.0%) had confirmed ASC-US by cytology, 265 (0.5%) refused colposcopy and/or biopsy and were excluded, and the remaining 2393 (4.5%) who underwent colposcopy and/or biopsy were finally enrolled in this study. The characteristics of patients with ASC-US are shown in Table 1. The mean age of the women at enrollment was 38.4±10.3 years (range, 21 to 73 years). Approximately 75.3% had completed middle school or higher education, 97.5% of those enrolled denied having a history of smoking, 68.4% denied having a history of drinking, 35.7% had more than 2 pregnancies, and 14.7% had more than 2 deliveries (Table 1).

Prevalence Rates of the Different HPV Genotypes Among Women with ASC-US According to Cervical Pathologic Severity

Table 2 contains data on the incident rates of the different HPV genotypes among women with ASC-US. The HR-HPV infection rate of ASC-US women aged 21–30 years was 51.2%, and the HR-HPV infection rate gradually decreased with age, with the lowest rate of 40.7% among ASC-US women > 65 years. The prevalence of HR-HPV among women with ASC-US was 47.6% (1139/2393). HPV52 was the most prevalent, having been detected in 329 women (13.7%), followed by HPV16 (226/2393, 9.4%), HPV58 (201/2393, 8.4%), HPV51 (103/2393, 4.3%) and HPV18 (88/12393, 3.7%).

The rate of HR-HPV infection increased with the severity of the pathological diagnosis. The prevalence rate of HR-HPV was 32.6% among women with normal pathology and 97.6% among women with CIN3 or cancer (Supplementary Table S1). Table 3 shows the distribution of different HR-HPV genotype models according to the histologic diagnosis of CIN2+ in women with ASC-US. The rate of HR-HPV infection (95.0%) in women with ASC-US with biopsy-confirmed CIN2+ was significantly higher than that in women with biopsy-confirmed negative for intraepithelial lesion or malignancy (NILM)/CIN1 (42.3%). Among the women with ASC-US and biopsy-confirmed CIN2+, the prevalence rates of HR-HPV genotypes were as follows: HPV16, 47.9%; HPV58, 21.1%; HPV52, 18.6%; HPV18,

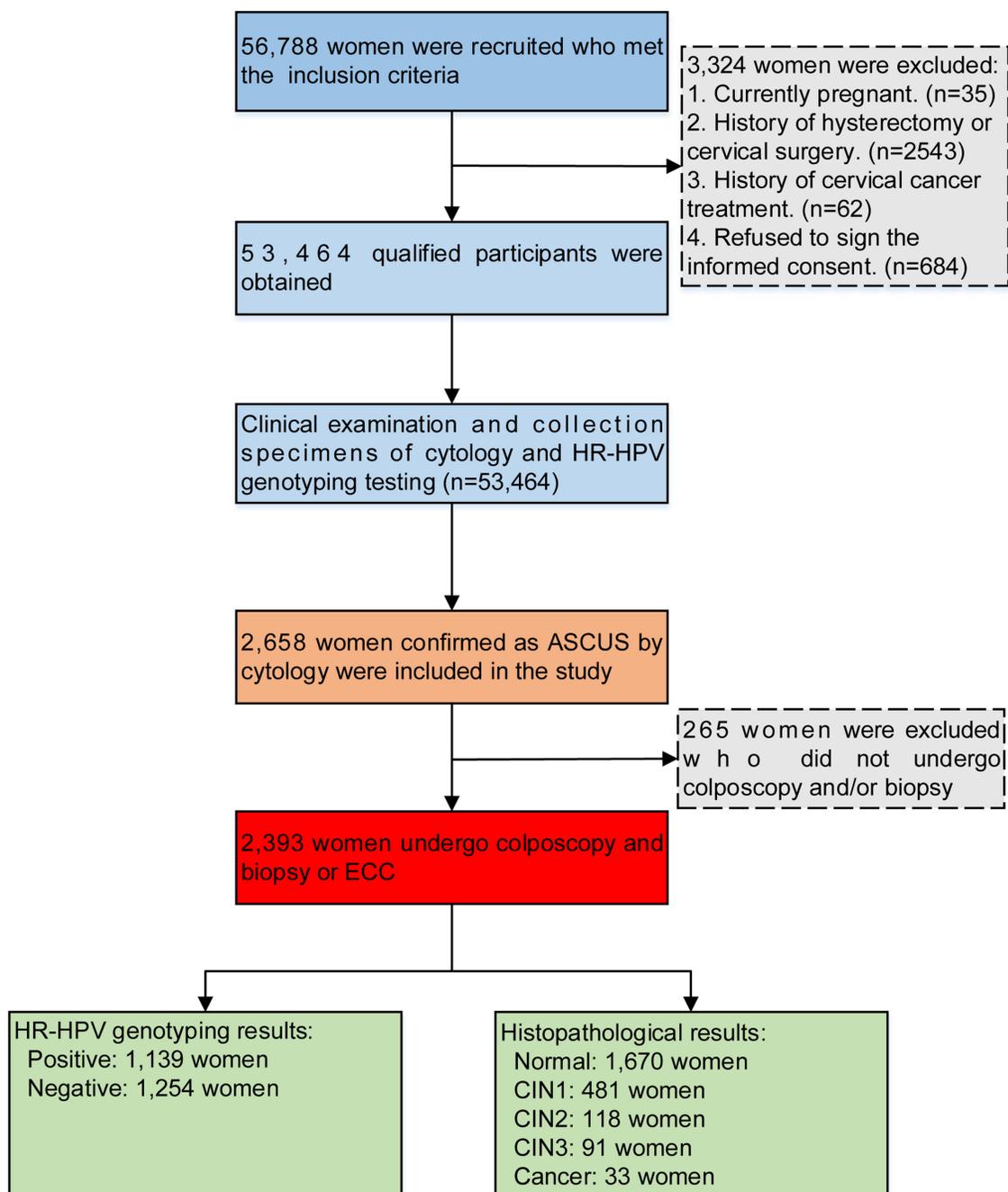


Figure 1 The flowchart of this study.

Abbreviations: HR-HPV, high-risk human papillomavirus, including types HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68; ASC-US, atypical squamous cells of undetermined significance; CIN1/2/3, cervical intraepithelial neoplasia grade 1/2/3; ECC, endocervical curettage.

7.0%; HPV33, 5.8%; HPV31, 5.4%; HPV56, 2.5%; HPV59, 2.5%; HPV39, 2.1%; HPV51, 2.1%; HPV45, 1.7%; HPV35, 1.2%; HPV66, 0.8% and HPV68, 0.8%. The prevalence rate of HR-HPV increased as more HPV genotypes were combined. The combination of HPV16/18/31/33/52/58 was able to identify 91.3% ($p < 0.001$) of women whose cytology was ASC-US and who had histologically proven CIN2+, similar

to the HR-HPV model (95.0%). However, based on the HPV16/18 model, the rate was only 54.1%.

Risk of CIN2+ Stratified by HR-HPV Genotype in Women with ASC-US

We analyzed the factors that may predict CIN2+ in women with ASC-US, as shown in [Table 4](#). HR-HPV-positive cases

Table 1 Characteristics for Women with Cytology Showing ASC-US (N=2393)

Characteristics	No. of Women	Mean (x±s) or Prevalence (%)
Age	2393	38.42±10.28
21–30	605	26.33±2.88
31–40	835	35.57±2.88
41–50	671	44.80±2.71
51–65	255	56.18±4.25
>65	27	70.56±3.52
Degree of education		
Uneducated	217	9.1
Primary school education	373	15.6
Middle school education	1188	49.6
≥Higher education	615	25.7
Drinking history		
Yes-At least 2 times a week	96	4.0
Yes- Less than 2 times a week	660	27.6
No	1637	68.4
Smoking history		
Yes-At least 2 times a week	49	2.0
Yes-Less than 2 times a week	12	0.5
No	2332	97.5
Times of pregnancy		
≤2	1538	64.3
>2	855	35.7
Times of childbirth		
≤2	2041	85.3
>2	352	14.7
HR-HPV infection		
Positive	1139	47.6
Negative	1254	52.4

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus, including types HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68.

were associated with 16.18 (95% confidence interval (CI), 7.87 to 33.24; $p<0.001$) times higher odds of histologic CIN2+ after adjustments for age, education level, smoking, drinking, number of pregnancies and number of childbirths. The risk was highest for CIN2+ among women with ASC-US with HPV16 infection (OR, 26.38; 95% CI, 18.28 to 38.09; $p<0.001$). HPV18 (OR, 4.44; 95% CI, 2.34 to 8.42; $p<0.001$), HPV31 (OR, 2.97; 95% CI, 1.37 to 6.45; $p=0.006$), HPV33 (OR, 3.38; 95% CI, 1.63 to 7.00; $p=0.001$), HPV52 (OR, 2.96; 95% CI, 1.96 to 4.48; $p<0.001$) and HPV58 (OR, 7.04; 95% CI, 4.59 to 10.78; $p<0.001$) were also associated with a higher risk of histologic CIN2+, whereas HPV35, HPV39, HPV45, HPV51, HPV56, HPV59, HPV66, and HPV68 did not have a significant effect on the risk of CIN2+. We further estimated

Table 2 Prevalence of Different HR-HPV Genotypes in Women with ASC-US (N=2393)

Variate	Positive n (%)	Negative n (%)
Age ^a		
21–30	310 (51.2%)	295 (48.8%)
31–40	392 (46.9%)	443 (53.1%)
41–50	314 (46.8%)	357 (53.2%)
51–65	112 (43.9%)	143 (56.1%)
>65	11 (40.7%)	16 (59.3%)
HR-HPV	1139 (47.6%)	1254 (52.4%)
HPV16	226 (9.4%)	2167 (90.6%)
HPV18	88 (3.7%)	2305 (96.3%)
HPV31	64 (2.7%)	2329 (97.3%)
HPV33	71 (3.0%)	2322 (97.0%)
HPV35	26 (1.1%)	2367 (98.9%)
HPV39	69 (2.9%)	2324 (97.1%)
HPV45	33 (1.4%)	2360 (98.6%)
HPV51	103 (4.3%)	2290 (95.7%)
HPV52	329 (13.7%)	2064 (86.3%)
HPV56	81 (3.4%)	2312 (96.6%)
HPV58	201 (8.4%)	2192 (91.6%)
HPV59	62 (2.6%)	2331 (97.4%)
HPV66	60 (2.5%)	2333 (97.5%)
HPV68	67 (2.8%)	2326 (97.2%)

Notes: ^aOnly consider the prevalence of HR-HPV.

Abbreviations: HR-HPV, high-risk human papillomavirus, including types HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68. ASC-US, atypical squamous cells of undetermined significance.

the risk of CIN2+ according to different HPV genotype models among women with ASC-US. The estimated ORs of CIN2+ were 19.34 (95% CI, 13.76 to 27.19; $p<0.001$) for the HPV16/18 model, 18.73 (95% CI, 13.46 to 26.07; $p<0.001$) for the HPV16/18/58 model, 16.99 (95% CI, 11.73 to 24.61; $p<0.001$) for the HPV16/18/52/58 model, 19.76 (95% CI, 13.22 to 29.55; $p<0.001$) for the HPV16/18/33/52/58 model, and 15.08 (95% CI, 7.61 to 29.86; $p<0.001$) for the HPV16/18/31/33/52/58 model.

Effectiveness of Different HR-HPV Genotype Models for the Triage of Women with ASC-US

The study endpoint was histological detection of CIN2+ at any of the follow-up visits. The sensitivities and NPVs of the different HR-HPV genotype models increased with the increase in the number of combined HPV genotypes, and the highest was the HR-HPV model followed by HPV16/18/31/33/52/58, HPV16/18/33/52/58, HPV16/18/52/58, HPV16/18/58 and HPV16/18. However, as the number of HPV genotype combinations increased, the specificity and

Table 3 Distribution of Different HR-HPV Genotypes Model According to Histologic Diagnosis in Women with ASC-US. (N=2393)

HR-HPV Types Model	Histologic Diagnosis		P-value
	Normal/CINI N=2151, n (%)	CIN2+ N=242, n (%)	
HR-HPV	909 (42.3%)	230 (95.0%)	<0.001
HPV16	110 (5.1%)	116 (47.9%)	<0.001
HPV18	71 (3.3%)	17 (7.0%)	0.004
HPV31	51 (2.4%)	13 (5.4%)	0.006
HPV33	57 (2.6%)	14 (5.8%)	0.006
HPV35	23 (1.1%)	3 (1.2%)	1.000
HPV39	64 (3.0%)	5 (2.1%)	0.423
HPV45	29 (1.3%)	4 (1.7%)	0.925
HPV51	98 (4.6%)	5 (2.1%)	0.070
HPV52	284 (13.2%)	45 (18.6%)	0.021
HPV56	75 (3.5%)	6 (2.5%)	0.411
HPV58	150 (7.0%)	51 (21.1%)	<0.001
HPV59	56 (2.6%)	6 (2.5%)	0.908
HPV66	58 (2.7%)	2 (0.8%)	0.078
HPV68	65 (3.0%)	2 (0.8%)	0.050
HPV16/18 ^a	178 (8.3%)	131 (54.1%)	<0.001
HPV16/18/58 ^b	314 (14.6%)	174 (71.9%)	<0.001
HPV16/18/52/58 ^c	568 (26.4%)	203 (83.9%)	<0.001
HPV16/18/33/52/58 ^d	611 (28.4%)	212 (87.6%)	<0.001
HPV16/18/31/33/52/58 ^e	645 (30.0%)	221 (91.3%)	<0.001

Notes: ^aWomen with HPV16 and/or HPV18 infection; ^ball women with any positive of HPV16, HPV18, and HPV58; ^call women with any positive of HPV16, HPV18, HPV52 and HPV58; ^dall women with any positive of HPV16, HPV18, HPV33, HPV52 and HPV58; ^eall women with any positive of HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58.

Abbreviations: HR-HPV, high-risk human papillomavirus, including types HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68; CIN, cervical intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; ASC-US, atypical squamous cells of undetermined significance.

PPV decreased. The sensitivity (91.3% vs 95.0%) and NPV (98.6% vs 99.0%) of the HPV16/18/31/33/52/58 model were similar to those of the HR-HPV model for identifying CIN2+ among women with ASC-US, but the specificity (70.0% vs 57.7%) and PPV (25.5% vs 20.2%) were higher than those of the HR-HPV model. In addition, the referral rate for the HPV16/18/31/33/52/58 model (36.2%) was significantly lower than that for the HR-HPV model (47.6%) (Table 5).

Discussion

ASC-US is the most frequent abnormal cytology result in cervical screening, but most patients do not present cervical

Table 4 Risk of CIN2+ Stratified by HR-HPV Genotype in Women with ASC-US (N=2393)

HR-HPV Types	No. of Women	OR	OR _{adjust} (95% CI) ^a	P-value
Age				
21–30	605	1 (R)	1 (R)	
31–40	835	1.62 (1.10–2.38)	2.01 (1.28–3.17)	0.003
41–50	671	2.16 (1.47–3.18)	2.68 (1.70–4.22)	<0.001
51–65	255	1.17 (0.67–2.04)	1.56 (0.82–2.96)	0.176
>65	27	1.10 (0.25–4.80)	1.29 (0.17–9.77)	0.805
HR-HPV				
Negative	1254	1 (R)	1 (R)	
Positive	1139	26.19 (14.57–47.08)	16.18 (7.87–33.24)	<0.001
HPV16				
Negative	2167	1 (R)	1 (R)	
Positive	226	17.08 (12.44–23.45)	26.38 (18.28–38.09)	<0.001
HPV18				
Negative	2305	1 (R)	1 (R)	
Positive	88	2.21 (1.28–3.82)	4.44 (2.34–8.42)	<0.001
HPV31				
Negative	2329	1 (R)	1 (R)	
Positive	64	2.34 (1.25–4.36)	2.97 (1.37–6.45)	0.006
HPV33				
Negative	2322	1 (R)	1 (R)	
Positive	71	2.26 (1.24–4.11)	3.38 (1.63–7.00)	0.001
HPV35				
Negative	2367	1 (R)	1 (R)	
Positive	26	1.16 (0.35–3.90)	2.09 (0.58–7.55)	0.262
HPV39				
Negative	2324	1 (R)	1 (R)	
Positive	69	0.69 (0.27–1.73)	1.23 (0.43–3.52)	0.699
HPV45				
Negative	2360	1 (R)	1 (R)	
Positive	33	1.23 (0.43–3.53)	1.32 (0.41–4.31)	0.642
HPV51				
Negative	2290	1 (R)	1 (R)	
Positive	103	0.44 (0.18–1.10)	0.53 (0.19–1.50)	0.233
HPV52				
Negative	2064	1 (R)	1 (R)	
Positive	329	1.50 (1.06–2.13)	2.96 (1.96–4.48)	<0.001
HPV56				
Negative	2312	1 (R)	1 (R)	
Positive	81	0.70 (0.30–1.63)	0.66 (0.23–1.89)	0.438
HPV58				
Negative	2192	1 (R)	1 (R)	
Positive	201	3.56 (2.51–5.06)	7.04 (4.59–10.78)	<0.001

(Continued)

Table 4 (Continued).

HR-HPV Types	No. of Women	OR	OR _{adjust} (95% CI) ^a	P-value
HPV59				
Negative	2331	1 (R)	1 (R)	
Positive	62	0.95 (0.41–2.23)	0.76 (0.29–2.02)	0.582
HPV66				
Negative	2333	1 (R)	1 (R)	
Positive	60	0.30 (0.07–1.24)	0.14 (0.02–0.85)	0.032
HPV68				
Negative	2326	1 (R)	1 (R)	
Positive	67	0.27 (0.07–1.10)	0.22 (0.05–1.02)	0.053
HPV16/18 ^b				
Negative	2084	1 (R)	1 (R)	
Positive	309	13.08 (9.73–17.58)	19.34 (13.76–27.19)	<0.001
HPV16/18/58 ^c				
Negative	1905	1 (R)	1 (R)	
Positive	488	14.97 (11.04–20.30)	18.73 (13.46–26.07)	<0.001
HPV16/18/52/58 ^d				
Negative	1622	1 (R)	1 (R)	
Positive	771	14.51 (10.16–20.71)	16.99 (11.73–24.61)	<0.001
HPV16/18/33/52/58 ^e				
Negative	1570	1 (R)	1 (R)	
Positive	823	17.81 (12.02–26.40)	19.76 (13.22–29.55)	<0.001
HPV16/18/31/33/52/58 ^f				
Negative	1527	1 (R)	1 (R)	
Positive	866	24.57 (15.56–38.81)	15.08 (7.61–29.86)	<0.001

Notes: ^aOR values were adjusted for age, education level, smoking, drinking, number of pregnancy, and number of childbirth; ^bwomen with HPV16 and/or HPV18 infection directly referred to colposcopy and biopsy; ^call women with any positive of HPV16, HPV18, and HPV58 were directly referred to colposcopy and biopsy; ^dall women with any positive of HPV16, HPV18, HPV52 and HPV58 were directly referred to colposcopy and biopsy; ^eall women with any positive of HPV16, HPV18, HPV33, HPV52 and HPV58 were directly referred to colposcopy and biopsy; ^fall women with any positive of HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 were directly referred to colposcopy and biopsy.

Abbreviations: OR, odds ratio; ASC-US, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus, including types HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68; R, reference.

lesions or are only diagnosed with CIN1 during follow-up, and less than 10% of women progress to CIN2/3 or cancer.⁸ Thus, deciding the appropriate clinical management strategy for women with ASC-US is a problem that needs a solution. HR-HPV testing has been included in the programs of

cervical cancer screening in recent years, which can both be used to triage patients with ASC-US^{2,17} and be a part of screening combined with the TCT. The current management strategy is that all ASC-US women with an HR-HPV-positive result are immediately referred for colposcopy/biopsy.¹⁸ Different HR-HPV genotypes are associated with different levels of risk for the progression of CIN to cervical cancer. The effectiveness of specific HR-HPV types in the triage of women with ASC-US may vary with the different combination of different HPV genotypes. Thus, it is meaningful to identify the specific HR-HPV genotype model with higher sensitivity and specificity and lower referral rates to triage women with ASC-US.

Previous reports^{13,19} mainly evaluated the performance of HR-HPV or HPV16/18 genotyping in triaging patients with ASC-US. Nevertheless, data on HR-HPV types other than HPV16 and HPV18 are rare and inconsistent. This study evaluated the triage effectiveness of all HR-HPV genotypes in women with ASC-US, and we discuss the triage efficiency of different HR-HPV genotype models (HR-HPV, HPV16/18, HPV16/18/58, HPV16/18/52/58, HPV16/18/31/52/58, HPV16/18/31/33/52/58) for patients with ASC-US from a Chinese population whose biopsies proved to be CIN2+. We found that the HPV16/18/31/33/58/52 model has a relatively high sensitivity, specificity, PPV, and NPV but a lower referral rate than the HR-HPV model or other HR-HPV genotype models.

The rate of ASC-US findings by cervical cytology ranged from 3% to 10% in previous studies.^{8,20-22} In line with these results, our research found that ASC-US accounted for 5.0% of all cytology results. However, the prevalence of HR-HPV among women with ASC-US was quite inconsistent in different studies. Although one study reported that the prevalence rate of HR-HPV among women with ASC-US was 41.7%,²³ another study reported a prevalence rate of 23%.²⁴ The prevalence rate of HR-HPV among patients with ASC-US was 47.6% in our research. Moreover, among patients with ASC-US whose biopsies proved to be CIN2+, the infection rates of HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 were 47.9%, 7.0%, 5.4%, 5.8%, 18.6% and 21.1%, respectively. These findings suggest a higher possibility of progression to high-grade CIN in patients with ASC-US who are simultaneously infected with HPV16, HPV18, HPV31, HPV33, HPV52 or HPV58, which implies the significance of specific HPV genotype detection.

Previous studies compared the accuracy of the HR-HPV test with repeated cytology for potential CIN2+ or CIN3+ in patients with ASC-US. The results indicated that the HR-HPV

Table 5 The Effect of Different HR-HPV Genotypes Model in Triage Women with ASC-US. (N=2393)

HR-HPV Genotypes Model	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Referral Rate ^a % (n/N)
HR-HPV ^b	95.0 (92.3–97.8)	57.7 (55.7–59.8)	20.2 (18.1–22.7)	99.0 (98.5–99.6)	47.6 (1139/2393)
HPV16/18 ^c	54.1 (47.9–60.4)	91.7 (90.6–92.9)	42.4 (36.5–47.5)	94.7 (93.3–95.3)	12.9 (309/2393)
HPV16/18/58 ^d	71.9 (66.2–77.6)	85.4 (83.9–86.9)	35.7 (31.1–39.6)	96.4 (95.1–96.9)	20.4 (488/2393)
HPV16/18/52/58 ^e	83.9 (79.3–88.5)	73.6 (71.7–75.5)	26.3 (22.9–29.1)	97.6 (96.2–97.8)	32.2 (771/2393)
HPV16/18/33/52/58 ^f	87.6 (83.5–91.8)	71.6 (69.7–73.5)	25.8 (22.5–28.5)	98.1 (96.7–98.2)	34.4 (823/2393)
HPV16/18/31/33/52/58 ^g	91.3 (87.8–94.9)	70.0 (68.1–72.0)	25.5 (22.4–28.2)	98.6 (97.3–98.7)	36.2 (866/2393)

Notes: ^aThe rate of referred to colposcopy in ASC-US women; ^ball women with any positive of HPV16, HPV18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66 and HPV68 were directly referred to colposcopy and biopsy; ^cwomen with HPV16 and/or HPV18 infection directly referred to colposcopy and biopsy; ^dall women with any positive of HPV16, HPV18, and HPV58 were directly referred to colposcopy and biopsy; ^eall women with any positive of HPV16, HPV18, HPV52 and HPV58 were directly referred to colposcopy and biopsy; ^fall women with any positive of HPV16, HPV18, HPV33, HPV52 and HPV58 were directly referred to colposcopy and biopsy; ^gall women with any positive of HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 were directly referred to colposcopy and biopsy. **Abbreviations:** HR-HPV, high-risk human papillomavirus, including types HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68; PPV, positive predictive value; NPV, negative predictive value.

test is more sensitive than repeated cytology in the triage of patients with ASC-US, but there is no significant difference in the specificity.² Our study shows that the sensitivity of HR-HPV to detect CIN2+ among patients with ASC-US is 95.0% but that the specificity is only 57.7%. Therefore, triaging patients with ASC-US with the recommended HR-HPV test will increase the referral rates of colposcope, unnecessary costs and burden to patients. In our previous study,²⁵ HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 were the most common genotypes in women with HSILs in China. Other studies²⁶ have also indicated that the prevalence rates of HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 among patients with ASC-US with detected CIN2 or CIN3 are higher. This is consistent with our research, and we found that HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 were the most prevalent among patients with ASC-US with a histologic diagnosis of CIN2+. Therefore, the risk of different HPV genotypes associated with CIN2+ among patients with ASC-US was estimated. We found that HR-HPV positivity was associated with an increased odds of CIN2+ histology (OR, 16.18; 95% CI, 7.87 to 33.24). In the analysis of different HR-HPV genotypes, the risk of CIN2+ was highest among women with HPV16 infections. HPV18, HPV31, HPV33, HPV52 and HPV58 were also associated with the risk of histologically proven CIN2+, whereas HPV35, HPV39, HPV45, HPV51, HPV56, HPV59, HPV66, and HPV68 did not have a significant effect on the risk. An increased risk for CIN2+ was observed when the model combined more genotypes. The estimated OR of HPV16/18/58/52/31/33 was 15.08 (7.61 to 29.86) according to our research.

Therefore, immediate colposcopy/biopsy of women with ASC-US who are positive for HPV16/18/58/52/31/33 may be an alternative strategy. We calculated the

sensitivity, specificity, PPV, and NPV of each HPV genotype combination model, including HPV16/18, HPV16/18/58, HPV16/18/52/58, HPV16/18/31/52/58, HPV16/18/31/33/52/58 and HR-HPV, to identify potential CIN2+ populations among patients with ASC-US. Similar to previous results,²⁷ HPV16/18 was much more specific than the HPV16/18/31/33/52/58 combination (91.7% vs 70.0%) but lost sensitivity (54.1% vs 91.3%), which implies a higher possibility of misdiagnosis. However, we found that the sensitivity of HPV16/18/31/33/52/58 for identifying CIN2+ in women with ASC-US was similar to that of the HR-HPV model but higher than that of HPV16/18/33/52/58, HPV16/18/52/58, or HPV16/18/58. The specificity of the HPV16/18/31/33/52/58 model was higher than that of the HR-HPV model (70.0% vs 57.7%). Moreover, according to the current guidelines for cervical cancer screening, HR-HPV-positive patients with ASC-US are immediately referred for colposcopy, and the referral rate of HPV16/18/31/33/52/58 was significantly lower than that recommended for HR-HPV (36.2% vs 47.6%), which can effectively reduce the high burden of colposcopy referrals. Therefore, it is reasonable to believe that the HPV16/18/31/33/52/58 genotype model is an alternative triage strategy to identify CIN2+ among patients with ASC-US in China.

Potential limitations are that only 1 case of cervical adenocarcinoma was identified in this study, and the study cannot conclude the role of HPV genotyping in the detection of cervical adenocarcinoma. This will impact conclusions regarding the importance of specific genotypes such as HPV45, which are relatively common in cervical adenocarcinomas but less common in CIN2/3 lesions.¹⁵ In addition, this research was conducted in a single region,

and we did not perform verification in other regions; thus, it may not be generalizable to other places. Last, this study was not based on a large-scale follow-up, which means it may not reflect the actual screening population.

Conclusion

This study evaluated the effectiveness of type-specific HR-HPV genotypes in the triage of patients with ASC-US in the largest population to date. Due to its high sensitivity and specificity and minimal number of HR-HPV genotypes, we suggest the HPV16/18/31/33/58/52 genotype model as an alternative strategy for the triage of patients with ASC-US. Developing non-16/18 HPV genotyping assays could significantly increase the cost effectiveness of triaging women with ASC-US. To validate our results, further work is needed to evaluate our novel HPV genotype model in external populations.

Abbreviations

HR-HPV, high-risk human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; CIN, cervical epithelial neoplasia; TCT, liquid-based cytology; DNA, deoxyribonucleic acid; ECC, endocervical curettage; PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio.

Ethics Approval

This study was conducted in accordance with the Declaration of Helsinki, and was approved by the Ethics Committees of the Fujian Maternity and Child Health Hospital (2014-045).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to

which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests for this work.

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