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Clinical significance of genotyping for human papillomavirus (HPV) 16 18/45 combined with cytology in cervical exfoliated cells in HPV oncogenic mRNA-positive women

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HIGHLIGHTS

- The performances of different triaging strategies for the greater sample size of AHPV-positive women were compared.
- The AHPV-GT test may be a promising triage approach in AHPV-positive women.
- Our results support combination AHPV-GT with cytology triaging for AHPV-positive women.

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ABSTRACT

Objectives. This study aimed to assess human papilloma virus (HPV) 16 18/45 typing test results combined with cytology for cervical exfoliated cells from women who screened positive in an HPV E6/E7 mRNA assay (Aptima HPV, AHPV).

Methods. In total, 3257 AHPV-positive women aged 25–65 years were underwent AHPV 16 18/45 Genotype assay (AHPV-GT) testing with cytology. Women were referred for colposcopy and further biopsy if indicated. Different triaging strategies were compared.

Results. Overall, 624 women (19.2%) tested AHPV-GT positive. When identifying CIN2+, compared with cytology, AHPV-GT achieved a similar AUC (0.72 vs. 0.69, $P = 0.158$) but a higher specificity (85.1% vs. 79.3%, $P < 0.001$) and positive predictive value (PPV) (29.6% vs. 23.2%, $P < 0.001$). When identifying CIN2+, compared with cytology, the cotesting strategy (cytology combined with AHPV-GT) increased the AUC from 0.69 to 0.76 ($P < 0.001$), with a higher sensitivity (84.6% vs. 59.5%, $P < 0.001$), higher NPV (97.6% vs. 94.9%, $P < 0.001$) and similar PPV (21.6% vs. 23.2%, $P = 0.054$). When identifying CIN2+, the results of combination strategy (AHPV-GT genotyping plus reflex cytology in women positive for the 11 other hrHPV genotypes) were consistent with those of the cotesting strategy. Similar results were achieved when identifying CIN3 + .

Conclusions. The AHPV-GT test may be a promising triage approach with high specificity in women receiving AHPV-positive primary screening tests. Although a combination strategy and cotesting strategy detected the same CIN2+ and CIN3+ cases, the former required significantly fewer screening tests.

1. Introduction

Cervical cancer is the third most common cancer among women worldwide, with >528,000 new cases and 266,000 deaths annually, and >85% of these cases occur in less developed countries [1]. Persistent infection with high-risk human papillomavirus (hrHPV) is a causal factor for cervical cancer [2,3]. The expression of the HPV E6 and E7 oncoproteins is necessary and sufficient for cervical cell immortalization, neoplastic transformation, and the development of invasive cancer [4]. HPV DNA tests, such as the Hybrid Capture 2 assay (HC2) HPV and Cobas 4800 HPV, are used alone as a primary cervical cancer screening tool in women aged 25 years or older in many countries [5,6]. In recent years, compared with DNA-based HPV tests, the hrHPV E6/E7 mRNA assay (Aptima HPV [AHPV]), has shown significantly higher specificity, has been associated with a greater positive predictive value (PPV) and has achieved a similar sensitivity for identifying cervical intraepithelial neoplasia (CIN) 2+ when used with cervical exfoliated cells [7–9]. The AHPV exhibits both high sensitivity and a higher specificity, reflecting its advantages as a tool for primary screening [10]. Therefore, the AHPV has been supported for use in cervical cancer primary screening

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[11]. Compared with HPV DNA-based tests, the AHPV test reduced false-positive results by 24% in a negative for intraepithelial lesions or malignancy (NILM) group, but its PPV for CIN2+ was only 6.3%–21.1% in cervical cancer primary screening [11,12]. Therefore, additional clinical data are needed in AHPV-positive women.

However, no previous report has explored a triaging method for primary cervical cancer screening in AHPV-positive women. Cytology is the most commonly used test in hrHPV-positive women [13], but the disadvantages of this method include poor interobserver agreement and the need for well-trained cytologists [14]. Therefore, new triage approaches for AHPV-positive women are urgently needed. HPV genotypes 16 and 18 were recommended for triaging Cobas HPV-positive women in the 2015 Society of Gynecologic Oncology (SGO)/American Society for Colposcopy and Cervical Pathology (ASCCP) interim clinical guidelines [15]. The Aptima HPV 16 18/45 Genotype (GT) assay (AHPV-GT) can detect HPV 16 and a subset of HPV 18 and 45 cases. The AHPV-GT is simple to use in cervical exfoliated cells, and the results of this tests are reproducible and do not require a well-trained cytologist. More importantly, the AHPV-GT has been reported to be suitable for triaging HC2-positive cases [16]. Thus, the AHPV-GT may be a proper triage method for AHPV-positive women.

In this study, we performed the AHPV-GT and cytology in cervical exfoliated cells obtained from 3257 AHPV-positive women and evaluated the performance of the AHPV-GT test combined with cytology in this clinical population.

2. Materials and methods

2.1. Study design

From October 2016 to October 2017, a total of 24,236 eligible women aged 25–65 years underwent cervical cancer screening in the physical examination center and gynecological clinic of Huzhou Maternity & Child Health Care Hospital, China. Of these individuals, 23,794 (98.2%) met the eligibility criteria. Women were excluded from the study according to the following criteria: 1) previously confirmed CIN, cervical cancer, or other malignancies; 2) a previous therapeutic procedure performed on the cervix, such as cervical microwave, electrocautery and other physical treatment or cervical conization; and 3) pregnancy. A total of 423 women were excluded because of a single AHPV test or liquid-based cytology (LBC), resulting in 23,371 eligible women for this analysis. A total of 3331 AHPV-positive women were detected among the 23,371 women and were selected for colposcopy and further biopsy was performed if indicated based on colposcopy algorithm: 50 of these women did not proceed to colposcopy, and 24 had missing cytology results. Therefore, valid data for all tests were available for 3257 women (97%) (Fig. 1).

2.2. AHPV testing and liquid-based cytology

A single cervical specimen was collected from each participant and suspended in PreservCyt collection medium (Hologic Inc., Marlborough, MA) according to the manufacturer's instructions. The AHPV and AHPV-GT (Gen-Probe Inc., San Diego, CA) were performed using an automated Panther System (Hologic, Inc., San Diego, CA) with residual PreservCyt samples (1-mL aliquots) obtained from the participants according to the manufacturer's instructions. The E6/E7 oncogenic mRNA, which is associated with 14 hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), was detected by the AHPV. AHPV-positive samples were reflex-tested with the AHPV-GT, which can detect the HPV E6/E7 mRNA in hrHPV genotypes 16 and 18/45. LBC was performed using a ThinPrep 2000 processor (Cytyc Corporation, Marlborough, MA), and the results were analyzed by cytologists in our hospital according to the 2014 Bethesda system [17]. Cytologists were masked to the AHPV and AHPV-GT test results until the cytology diagnosis was completed.

2.3. Colposcopy and histological diagnosis

Colposcopy was performed for all the women by a professional gynecologist in a colposcopy clinic. A woman underwent combined cervical biopsy with endocervical curettage (ECC) if she presented with one of the following conditions: a high-grade cytology result (a high-grade squamous intraepithelial lesion [HSIL], atypical squamous cells that could not exclude HSIL [ASC-H] or squamous cell carcinoma [SCC]), low-grade cytology result(atypical squamous cells of undetermined significance [ASC-US] or a low-grade squamous intraepithelial lesion [LSIL]) without visible lesions; atypical glandular cells (AGCs), an AHPV-GT+ result, unsatisfactory colposcopy, or visible lesions. Women with a low-grade cytology result other than those described above underwent only cervical biopsy. Women with an NILM cytology result in whom satisfactory colposcopy, AHPV-GT-negative and no lesions were observed did not undergo cervical biopsy and ECC, and their histological diagnoses were considered normal on ethical grounds. Colposcopists were made aware of the cytology and AHPV-GT results before the colposcopy visit was performed. A histological diagnosis was obtained from at least 2 pathologists in the hospital and based on the 2014 World Health Organization (WHO) Classification of Tumors of the Female Genital Tract [18]. $P16^{INK4A}$ immunohistochemistry was utilized to adjudicate any CIN2 interpretation. If a disagreement regarding a histological diagnosis occurred, then a discussion was held by all pathologists in our hospital until a consensus was reached.

2.4. Statistical analysis

SPSS Statistics 19.0 (IBM Corp., Armonk, New York, USA) and R software 3.5.1 (Vienna, Austria) were used in this study. A P value <0.05 (two-sided) was considered statistically significant. Categorical variables were presented as the frequency (proportion). The sensitivity, specificity, PPV, negative predictive value (NPV) and area under the curve (AUC) were determined when comparing different diagnostic tests. The 95% confidence intervals (CIs) of proportions were calculated based on the following equation: $p \pm 1.96\sqrt{p(1-p)/n}$, where n is the number of cases involved in the calculation for proportion. The Cochran-Armitage trend test was used to study the underlying trends for the type of cytology or histology and the positive genotype rate. Pearson's Chi-Square or Fisher's exact probability test was used to compare performance efficiency between the AHPV and LBC assays. Sensitivity and specificity were compared using McNemar's test for paired data, while a weighted generalized score statistic for comparisons of predictive values of diagnostic tests was used to compare PPVs and NPVs [19].

3. Results

3.1. Participant disposition and demographic information

As shown in Fig. 1, the median age of the 3257 enrolled women was 40.15 ± 9.72 years. All of the women were diagnosed based on cytology, including NILM (n = 2461), ASC-US (n = 342), LSIL (n = 316), ASC-H (n = 45), HSIL (n = 82), SCC (n = 9), atypical glandular cells-not otherwise specified (AGC-NOS) (n = 1), and atypical glandular cells-neoplastic (AGC-NEO) (n = 1). Histologic examinations revealed that of the included women, 2620 had normal findings (815 AHPV-GT negative, NILM cytology, and colposcopy(−) women were regarded as histologically normal on ethical grounds), 326 had CIN1, 46 had CIN2, 159 had CIN3, 2 had adenocarcinoma (ADC) in situ, 27 had SCC, 7 had ADC and 1 had adenosquamous carcinoma (ASC). Among the AHPV-positive women, 9.7% (317/3257) were diagnosed with CIN2+, and 6.1% (200/3257) were diagnosed with CIN3+.

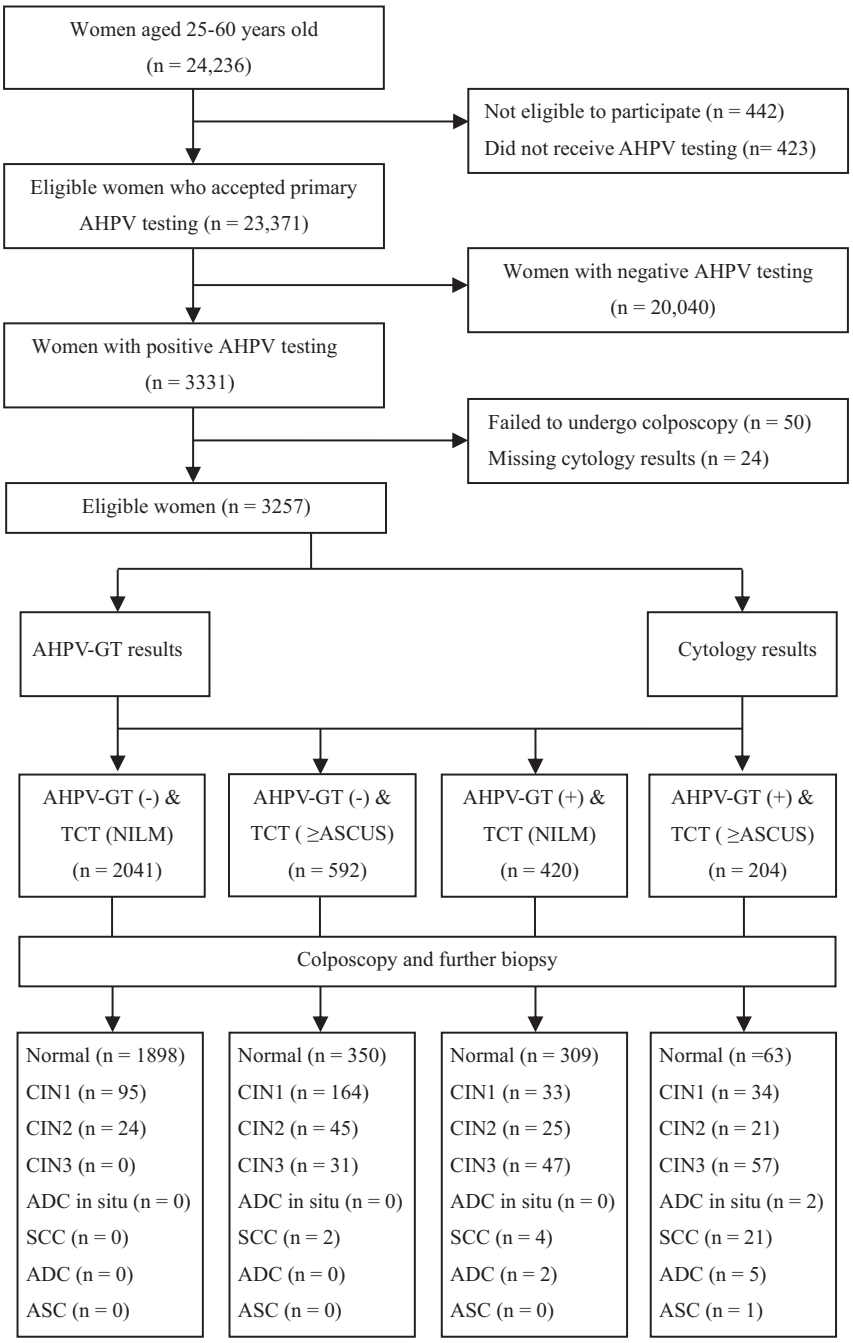

Fig. 1. Management procedure, results and outcomes. AHPV, Aptima human papillomavirus assay; AHPV-GT, Aptima HPV 16 18/45 Genotype assay; NILM, negative for intraepithelial lesion or malignancy; ≤ASC-US, abnormal squamous cells of uncertain significance or better; ≥ASC-US, abnormal squamous cells of uncertain significance or worse; ≥LSIL, low-grade squamous intraepithelial lesion or worse; CIN, cervical intraepithelial neoplasia; ADC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma.

3.2. AHPV-GT and cytology prevalence

The AHPV-GT, HPV16 and HPV18/45 results according to cytology results are shown in Table 1. Overall, 624 women (19.2%) tested AHPV-GT positive; of these women, 425 (13.0%), 194 (6.0%) and 5 (0.2%) tested HPV16+/18/45−, HPV16−/18/45+ and HPV16+/18/45 +, respectively. The positive rates of AHPV-GT and HPV16 increased with cytology grading in squamous epithelium (NILM, ASC-US, LSIL, ASC-H, HSIL and SCC) (Cochran-Armitage trend test $Z = 9.24$ and $Z = 9.95$, respectively; $P < 0.001$). The positive rate of AHPV 18/45 did not increase with cytology grading in squamous epithelium (Cochran-Armitage trend test $Z = 0.70$, $P > 0.05$).

3.3. AHPV-GT and disease prevalence

The AHPV-GT, HPV16 and HPV18/45 test results are shown in Table 2. According to histology results the positive rates of the AHPV-GT and HPV16 increased with histology grading in squamous epithelium (normal cervical tissue, CIN1, CIN2, CIN3 and SCC) (Cochran-Armitage trend test $z = 20.22$ and $z = 21.35$, respectively; $P < 0.001$). The positive rate of HPV18/45 did not increase with histology grading in squamous epithelium (Cochran-Armitage trend test $z = 1.93$; $P > 0.05$). The positive rate of HPV16 was significantly higher in the CIN2+ group than in the CIN1− group (53.7% [167/311] vs. 8.9% [263/2946], $P < 0.001$). The positive rate of HPV18/45 was slightly higher in the CIN2+ group than

Table 1
AHPV-GT, HPV16 and HPV18/45 tests results according to cytology results.

Cytology	Genotype Status, n (%)		Genotype Positive, n (%)		
	AHPV-GT (+)	AHPV-GT (−)	AHPV-GT (+)		
			16+, 18/45−	16−, 18/45+	16+, 18/45+
NILM (n = 2461)	420 (17.1)	2041 (82.9)	272 (11.1)	147 (6.0)	1 (0.0)
ASC-US (n = 342)	63 (18.4)	279 (81.6)	44 (12.9)	18 (5.3)	1 (0.3)
LSIL (n = 316)	65 (20.6)	251 (79.4)	46 (14.6)	17 (5.4)	2 (0.6)
ASC-H (n = 45)	23 (51.1)	22 (48.9)	19 (42.2)	4 (8.9)	0 (0)
HSIL (n = 82)	45 (54.9)	37 (45.1)	38 (46.3)	6 (7.3)	1 (1.2)
SCC (n = 9)	7 (77.8)	2 (22.2)	5 (55.6)	2 (22.2)	0 (0)
AGC-NOS (n = 1)	0(0)	1 (100.0)	0 (0)	0 (0)	0 (0)
AGC-NEO (n = 1)	1 (100.0)	0 (0)	1 (100.0)	0 (0)	0 (0)
Total (n = 3257)	624 (19.2)	2633 (80.8)	425 (13.0)	194 (6.0)	5 (0.2)
z	9.24		9.95	0.70	3.15
P	<0.001		<0.001	0.486	0.002

Abbreviations: AHPV, Aptima human papillomavirus assay; AHPV-GT, Aptima HPV 16 18/45 genotype assay; NILM, negative for intraepithelial lesion or malignancy; ASC-US, abnormal squamous cells of uncertain significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cell cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGC-NOS, atypical glandular cells not otherwise specified; AGC-NEO, atypical glandular cells-neoplastic.
The Cochran-Armitage trend test was used to study the underlying trend.

in the CIN1− group (7.4% [23/311] vs. 14.9% [176/2946], $P = 0.320$). The positive rate of the AHPV-GT was significantly higher in the CIN2+ group than in the CIN1- group (59.5% [185/311] vs. 14.9% [439/2946], $P = 0.000$). The positive rates of HPV16 and HPV18/45 were both significantly higher in the CIN3+ group than in the CIN2- group (63.8% [125/196] vs. 10.0% [305/3061] and 9.7% [19/196] vs. 5.9% [180/3061], $P < 0.001$ and $P = 0.031$, respectively).

3.4. Impact of age on AHPV status, CIN2+, and CIN3+

To determine the impact of the AHPV-GT at different ages, the prevalence of AHPV-GT positivity and cytological abnormalities were compared, as shown in Table 3. The number of AHPV-GT-positive cases in the 40–49-year-old group was 1072, which was the highest number among all groups. Although the women in this group accounted for 30.9% (95% CI; 27.4–34.7%) of all study subjects, 41.2% (95% CI; 35.7–46.9%) and 41.8% (95% CI; 34.9–49.1%) tested CIN2+ and CIN3+, respectively. In all age groups, the AHPV-GT positive rates (70.6%; 95% CI; 56.0–82.1% and 83.3%; 95% CI; 64.5–93.7%, respectively) of the CIN2+ and CIN3+ women in the 25–29-year-old group were the highest. The rates of cytological abnormalities (75.0%; 95% CI; 57.5–87.3% and 73.1%; 95% CI; 51.9–87.6%, respectively) of the CIN2+ and CIN3+ women in the 50–59-year-old group were the highest.

Table 2
AHPV-GT, HPV16 and HPV18/45 tests results according to histology results.

Histology	Genotype Status, n (%)		Genotype Positive, n (%)		
	AHPV-GT (+)	AHPV-GT (−)	AHPV-GT (+)		
			16+, 18/45−	16−, 18/45+	16+, 18/45+
Normal (n = 2620)	372 (14.2)	2248 (85.8)	222 (8.5)	150 (5.7)	0 (0)
CIN1 (n = 326)	67 (20.6)	259 (79.4)	41 (12.6)	26 (8.0)	0 (0)
CIN2 (n = 115)	46 (40.0)	69 (60.0)	42 (36.5)	4 (3.5)	0 (0)
CIN3 (n = 159)	104 (65.4)	55 (34.6)	95 (59.7)	5 (3.1)	4 (2.5)
ADC in situ (n = 2)	2 (100.0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)
SCC (n = 27)	25 (92.6)	2 (7.4)	20 (74.1)	5 (18.5)	0 (0)
ADC (n = 7)	7 (100.0)	0 (0)	4 (57.1)	2 (28.6)	1 (14.3)
ASC (n = 1)	1 (100.0)	0 (0)	0 (0)	1 (100.0)	0 (0)
Total (n = 3257)	624 (19.2)	2633 (80.8)	425 (13.0)	194 (6.0)	5 (0.2)
z	20.22		21.35	1.93	7.83
P	<0.001		<0.001	0.053	<0.001

Abbreviations: AHPV, Aptima human papillomavirus assay; AHPV-GT, Aptima HPV 16 18/45 genotype assay; CIN, cervical intraepithelial neoplasia; ADC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma.
The Cochran-Armitage trend test was used to study the underlying trend.

3.5. Comparison of different screening strategies

The performances of different screening strategies used to detect cervical disease are shown in Table 4. When identifying CIN2+, compared with cytology, the AHPV-GT achieved a similar AUC (0.72 [95% CI; 0.69–0.75] vs. 0.69 [95% CI; 0.66–0.72], $P = 0.158$), a higher specificity (85.1% [95% CI; 83.7–86.4%] vs. 79.3% [95% CI; 77.7–80.7%], $P < 0.001$) and PPV (29.6% [95% CI; 26.1–33.4%] vs. 23.2% [95% CI; 20.4–26.4%], $P < 0.001$) and the same sensitivity (59.5% [95% CI; 53.8–64.9%]). When identifying CIN2+, compared with cytology, the cotesting strategy (cytology combined with the AHPV-GT) increased the AUC from 0.69 to 0.76 ($P < 0.001$), with a higher sensitivity (84.6% [95% CI; 80.0–88.3%] vs. 59.5% [95% CI; 53.8–64.9%], $P < 0.001$) and NPV (97.6% [95% CI; 96.9–98.2%] vs. 94.9% [95% CI; 93.9–95.7%], $P < 0.001$). When identifying CIN2+, the results of combination strategy (AHPV-GT genotyping plus reflex cytology in women positive for the 11 other hrHPV genotypes) were consistent with those of the cotesting strategy. Similar results were achieved when identifying CIN3+. Compared with the AHPV primary and cotesting strategies, in the combination strategy, the number of colposcopy examinations required by the combination and cotesting strategies were reduced to 37.3% (1216/3257) in this study. Compared with the cotesting strategy, in the combination strategy, the number of women needing LBC was reduced to 80.8% (2633/3257).

4. Discussion

As E6/E7 mRNA expression occurs in only actively infected cells and gross transcript levels increase during CIN development and progression. The mRNA-based AHPV test has been found be more specific than DNA-based HPV tests in detecting high-grade disease [20,21] Therefore, the AHPV test is more suitable for primary screening of cervical cancer [10,20]. Because it has a low PPV for CIN2+ detection (from 6.3% to 21.1%) [11,12], referring all AHPV-positive women for colposcopy will result in overdiagnosis, overtreatment and high costs. Therefore, a triage strategy is needed for AHPV-positive women. However, no previous report has presented a strategy to manage AHPV-positive women. In the CLEAR study, women with AHPV-negative result had very low cervical disease risk after 3 years of follow-up (<0.3%) suggesting that a 3-year interval after AHPV-negative result could be considered acceptable [21,22]. Nevertheless, the best screening interval is still unknown.

HPV genotypes 16, 18 and 45 are more likely than other HPV types to be integrated into the human genome [23] and account for approximately 80% of all invasive cervical cancers worldwide [24]. Therefore, recommendations indicate that type-specific hrHPV DNA-based screening

Table 3
Impact of age on AHPV-GT status, CIN2+ and CIN3+.

	25–29 years	30–39 years	40–49 years	50–59 years	60–65 years	Total
Number	597	993	1072	510	85	3257
(% of row; 95% CI)	(18.3%; 17.0–19.7%)	(30.5%; 28.9–32.1%)	(32.9%; 31.3–34.6%)	(15.7%; 14.4–17.0%)	(2.6%; 2.1–3.2%)	
AHPV-GT (+)	110	186	193	93	25	624
(% of age group; 95% CI)	(17.6%; 14.8–20.9%)	(29.8%; 26.3–33.6%)	(30.9%; 27.4–34.7%)	(14.9%; 12.3–18.0%)	(4.0%; 2.7–5.9%)	
Cytology ≥ASC-US	104	253	263	128	25	796
(% of age group; 95% CI)	(13.1%; 10.8–15.7%)	(31.8%; 28.6–35.2%)	(33.0%; 29.8–36.4%)	(16.1%; 13.6–18.9%)	(3.1%; 2.1–4.7%)	
Number CIN2	21	37	44	10	3	115
(% of row; 95% CI)	(18.3%; 11.9–26.8%)	(32.2%; 23.9–41.6%)	(38.3%; 29.5–47.8%)	(8.7%; 4.5–15.8%)	(2.6%; 0.7–8.0%)	
Number CIN2 +	51	90	126	36	8	311
(% of row; 95% CI)	(16.4%; 12.6–21.1%)	(28.9%; 24.0–34.4%)	(41.2%; 35.7–46.9%)	(11.6%; 8.3–15.8%)	(2.6%; 1.2–5.2%)	
% Cytology ≥ASC-US	54.9%	63.3%	54.8%	75.0%	50.0%	
(95% CI) in CIN2+	(40.5%–68.6%)	(52.5%–73.1%)	(45.7%–63.6%)	(57.5%–87.3%)	(17.4%–82.6%)	
% AHPV-GT (+)	70.6%	54.4%	61.1%	52.8%	50.0%	
(95% CI) in CIN2+	(56.0%–82.1%)	(43.6%–64.9%)	(52.0%–69.5%)	(35.7%–69.2%)	(17.4%–82.6%)	
Number CIN3 +	30	53	82	26	5	196
(% of row; 95% CI)	(15.3%; 10.7–21.3%)	(27.0%; 21.1–33.9%)	(41.8%; 34.9–49.1%)	(13.3%; 9.0–19.0%)	(2.6%; 0.9–6.2%)	
% Cytology ≥ASC-US	56.7%	62.3%	57.3%	73.1%	60.0%	
(95% CI) in CIN3+	(37.7%–74.0%)	(47.9%–74.9%)	(45.9%–68.0%)	(51.9%–87.6%)	(17.0%–92.7%)	
% AHPV-GT (+)	83.3%	67.9%	69.5%	65.4%	80.0%	
(95% CI) in CIN3+	(64.5%–93.7%)	(53.6%–79.7%)	(58.2%–78.9%)	(44.4%–82.1%)	(29.9%–98.9%)	

AHPV, Aptima human papillomavirus assay; AHPV-GT, Aptima HPV 16 18/45 Genotype assay; ≥ASC-US,abnormal squamous cells of uncertain significance or worse; CIN, cervical intraepithelial neoplasia.

testing should focus on these HPV types [24]. It has been reported that the positive rate of HPV16 and HPV18 in invasive cervical cancer in Asia is 58.7%, which is significantly lower than the rates of 77.0% in North America and 73.5% in Europe [25]. HPV18 is only in the 6th position with a positive rate of 6.0% in HSIL among Chinese women [26], which is similar to our results. Therefore, the positive rate of the AHPV-GT for cytological diagnosis in the HSIL group is slightly lower, 54.9%, mainly due to different subtypes of HPV infection in different races and different regions.

In the CLEAR study, the risk of CIN2+ was 29.1% in AHPV-GT-positive women and 14.3% in other hrHPV-positive women with an ASC-US cytology diagnosis [27]. Although AHPV-GT positivity accounted for 19.2% of the included AHPV-positive women, the AHPV-GT still identified 58.4% of CIN2+ women and 70.9% of CIN3+ women, similar to previous reports [16,28].

Among all age groups, the AHPV-GT positive rate was highest for CIN2+ and CIN3+ women in the 25–29-year-old group. Wright et al. [29] found a substantial burden of CIN3+ in 25–29-year-old women

Table 4
Performances of different screening strategies for the detection of cervical disease.

	AUC(95% CI)	*P*	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
			n/N (%, 95% CI)	*P*	n/N (%, 95% CI)	*P*	n/N (%, 95% CI)	*P*	n/N (%, 95% CI)	*P*
To identify CIN2+										
Combination strategy	0.76(0.74, 0.78)	<0.001	263/311 (84.6%, 80.0–88.3)	<0.001	1993/2926 (67.7%, 65.9–69.3)	<0.001	263/1216 (21.6%, 19.4–24.1)	0.054	1993/2041 (97.6%, 96.9–98.2)	<0.001
Cotesting strategy	0.76(0.74, 0.78)	<0.001	263/311 (84.6%, 80.0–88.3)	<0.001	1993/2926 (67.7%, 65.9–69.3)	<0.001	263/1216 (21.6%, 19.4–24.1)	0.054	1993/2041 (97.6%, 96.9–98.2)	<0.001
AHPV-GT	0.72(0.69, 0.75)	0.158	185/311 (59.5%, 53.8–64.9)	1.001	2507/2946 (85.1%, 83.7–86.4)	<0.001	185/624 (29.6%, 26.1–33.4)	<0.001	2507/2633 (95.2%, 94.3–96.0)	0.477
Cytology	0.69(0.66, 0.72)	Reference	185/311 (59.5%, 53.8–64.9)	Reference	2335/2946 (79.3%, 77.7–80.7)	Reference	185/796 (23.2%, 20.4–26.4)	Reference	2335/2461 (94.9%, 93.9–95.7)	Reference
To identify CIN3+										
Combination strategy	0.77(0.74, 0.79)	<0.001	172/196 (87.8%, 82.1–91.8)	<0.001	2017/3061 (65.9%, 64.2–67.6)	<0.001	172/1216 (14.1%, 12.3–16.3)	0.257	2017/2041 (98.8%, 98.2–99.2)	<0.001
Cotesting strategy	0.77(0.74, 0.79)	<0.001	172/196 (87.8%, 82.1–91.8)	<0.001	2017/3061 (65.9%, 64.2–67.6)	<0.001	172/1216 (14.1%, 12.3–16.3)	0.257	2017/2041 (98.8%, 98.2–99.2)	<0.001
AHPV-GT	0.78(0.74, 0.81)	<0.001	139/196 (70.9%, 63.9–77.1)	0.033	2576/3061 (84.2%, 82.8–85.4)	<0.001	139/624 (22.3%, 19.1–25.8)	<0.001	2576/2633 (97.8%, 97.2–98.3)	0.007
Cytology	0.69(0.66, 0.73)	Reference	119/196 (60.7%, 53.5–67.5)	Reference	2384/3061 (77.9%, 76.4–79.3)	Reference	119/796 (14.9%, 12.6–17.7)	Reference	2384/2461 (96.9%, 96.1–97.5)	Reference

Abbreviations: combination strategy, combined genotyping for AHPV-GT plus reflex cytology in women positive for the 11 other high-risk HPV genotypes; cotesting strategy, cytology combined with AHPV-GT testing; AHPV-GT, Aptima HPV 16 18/45 Genotype assay; CIN, cervical intraepithelial neoplasia.
The *P*-values reported in the table are the results of comparison with the cytological triage method as a reference.
Sensitivities were compared using McNemar's test in CIN2+ or CIN3+ samples.
Specificities were compared by using a McNemar's test in CIN2- and CIN3- sample.
The positive predictive value and negative predictive value were compared by a weighted generalized score statistic for comparison of predictive values of diagnostic tests.

and as previously reported, that cytology was insensitive in this age group [30]. In this study, compared with cytology, the AHPV-GT, when used in triaging AHPV-positive women, has produced significantly higher specificity, been associated with more PPV and achieved a similar sensitivity for identifying CIN2+. These data indicate that AHPV-GT-positive women should be immediately referred for colposcopy [16] and that the AHPV-GT may represent a good technique for triaging AHPV-positive women.

In this study, the positive rates of HPV16 increased with higher histology and cytology grades in squamous epithelium, demonstrating that HPV16 was the main cause of cervical squamous cell lesions. The positive rates of HPV16 in SCC and ADC were 61.6% and 57.1%, respectively, similar to the results of Vinokurova et al. [23]. However, the positive rate of HPV18/45 was not correlated with histology or cytology grades in squamous epithelium. The positive rates of HPV18/45 were slightly higher in the CIN2+ group than in the CIN1- group. The above results suggest that CIN2+ is significantly associated with HPV16 but not HPV18/45 infection.

In this study, 2 cases of ADC in situ, 7 cases of ADC and 1 case of ASC were included. They accounted for 3.2% (10/311) of CIN2+, which was similar to 3.9% (17/431) in the ATHENA study [31]. It indicated that the roles of HPV18 and HPV45 in cervical adenocarcinoma screening would also be measured by this study. Currently, no effective screening method to predict whether CIN3 in older women is more likely to progress to cervical cancer has been identified. Some defects in cervical adenocarcinoma screening exist in all screening programs.

Current studies aiming to improve the triaging of AHPV-GT-negative women are very rare. Guo et al. [16] found that viral load was not suitable for further triaging AHPV-GT-negative women. Cytology was recommended for triaging women with hrHPV genotypes other than HPV16/18 in the 2015 SGO/ASCCP interim clinical guidelines [15]. The sensitivity of cytology was significantly increased for triaging versus screening [32,33]. In this study, we found that the positive rate of cytology ≥ ASC-US was significantly higher in the CIN2+ group than that in the CIN1- group (61.9% vs. 20.5%, $P < 0.001$) for AHPV-GT-negative women, suggesting that this cytological parameter has good triaging value in AHPV-GT negative women. Therefore, we next assessed the performance of the AHPV-GT test combined with cytology in cervical exfoliated cells obtained from AHPV-positive women.

Each strategy is associated with a trade-off between programmatic sensitivity and specificity. Although its higher specificity was confirmed, cytology also has disadvantages, such as its lower sensitivity, high inter-observer variability, higher false-negative/-positive rates and the requirement for more well-trained cytologists. More importantly, the cotesting strategy increased the cost of screening and the workload of medical staff, making it unsuitable for promotion and application in developing countries. A triage strategy is considered feasible if its NPV is equal to or exceeds a predefined threshold of 98% [34]. Of the four triage strategies evaluated here, the combination strategy and the cotesting strategy detected the same CIN2+ and CIN3+ cases and met the NPV threshold criterion of 98%. Compared with the cotesting strategies, the number of women who needed LBC and colposcopy in the combination strategy reduced to 80.8% and 37.3%, respectively. We acknowledge that the imperfect sensitivity (84.6%) of the combination triage strategy will result in some CIN2+ women failing to undergo immediate colposcopy, thus delaying their treatment. This risk will be significantly reduced if we have a short-term (for example, 12 months) recall for AHPV-positive/AHPV-GT-negative women. However, the normal screening interval is still unknown. Although sending all hrHPV-positive women for immediate colposcopy would eliminate this concern, this approach would require excessive referrals to colposcopy and treatment of some CIN2 patients exhibiting regression [35].

This study has several limitations. First, our data were derived from a clinic-based population rather than a general population and may therefore suffer biases, such as a higher positive rate on the AHPV test and the positive rate of 17.1% for the AHPV-GT in NILM women. Second, as a screening test, cytology was used to triage women who tested positive on AHPV primary screening, which may lead to underestimation of cytological performance. Third, although AHPV-GT positivity is a high-risk factor for CIN2+, the AHPV-GT test cannot determine whether the infection is very old or CIN2+ already exists.

The AHPV-GT test may be a promising triage approach with higher specificity in women receiving AHPV-positive primary screening tests. Although a combination strategy detected the same CIN2+ and CIN3+ cases detected by the cotesting strategy, the former required significantly fewer screening tests.

Conflict of interest statement

None of the authors declare any conflict of interest.

Role of the funding source

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Author contribution statement

J.W. and P.H. conceived and designed the study, and J.W. obtained funding. J.W., Y.D., J.D., Y.Z., P.W., X.Z. and Y.C. performed the clinical and experimental work related to cervical cancer screening. J.W. and Y.Z. analyzed and interpreted the experimental results. J.W. and D.N. performed the data analyses and wrote the initial draft with further input from all authors. J.W. and P.H. reviewed and edited the manuscript. All authors read and approved the final manuscript. All researchers listed as authors are independent from the funders, and all final decisions about the research were made without constraint by the investigators. J.W. had full access to all the data in the study and final responsibility for the decision to submit for publication.

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