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ORIGINAL ARTICLE

Clinical Performance of APTIMA Human Papillomavirus (HPV) 16 18/45 mRNA Genotyping Testing for the Detection of Cervical Intraepithelial Neoplasia 3 (CIN3) or Cancer in a Select Group of Chinese Women

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Abstract HPV type was evaluated in a select group of Chinese women that were positive with hybrid capture, and correlations were performed between the pathology found, the type of virus and a semiquantitation from the hybrid capture results. Totally 394 referred high-risk-HPV-positive women evaluated by Hybrid Capture 2 (HC-2) assay were enrolled. Before colposcopy, cervical specimens were collected from all participants and suspended into PreservCytcollection medium (Hologic Inc., Marlborough, MA), and tested with the APTIMA HPV16 18/45 mRNA assay. Colposcopy and diagnostic biopsies were done on all participants. Viral load was assessed by HC2 assay. Totally 55 women were diagnosed as CIN 3 plus cancer (≥CIN3), and the prevalence of HPV16/18/45 was 65.5 % (95 % confidence interval [CI], 52.9–78.0 %) among these ≥CIN3 women. Compared with the group with positive HC2 but negative HPV16/18/45, the odds ratio (OR) to identify ≥CIN3 was 6.3 (95 % CI, 3.2–12.3) for HPV16 and 3.2 (95 % CI, 1.4–7.2) for HPV18/45. When using ≥CIN3 as an endpoint, the sensitivity and specificity was 65.5 % (95 % CI, 52.9–78.0 %) and 72.0 % (95 % CI, 67.2–76.8 %). In the case of HPV16/18/45 negative, no high HPV load had a statistically significant increased risk for the prevalence of ≥CIN3. HPV16, 18 and 45 infection is a major cause for ≥CIN3 in Chinese women. Women with positive HPV16/18/45 should be referred to colposcopy immediately. HPV load was not suitable for the further triaged of the HPV16/18/45 negative cases.

Keywords Human papillomavirus · HPV mRNA genotyping · Cervical cancer · Screening

Highlights
1. HPV-positive women with HPV16, 18 and 45 have a substantial risk of cervical (pre-)cancer.
2. HPV16 18/45-mRNA is essential as a prognostic marker for ≥CIN3.
3. The APTIMA HPV16 18/45 mRNA assay is suitable for the triaged of the HC2 positive cases.

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Introduction

Persistent infection with high-risk human papillomavirus (hrHPV) is responsible for the progression of cervical precancerous lesions and invasive cervical cancer, and 99.7 % of cervical cancer cases worldwide are associated with certain types of HPV [1–3]. Studies [4–6] have shown that HPV primary screening is more sensitive than cytology and identifies cervical intraepithelial neoplasia (CIN) grade 3 or cervical cancer (≥CIN3). Currently in the United States (U.S.) and Europe have now adopted HPV testing as a stand-alone primary screening test for cervical cancer screening [7–9].

There are several HPV tests available in U.S. and Europe. Totally 37 high and low-risk HPV types have been identified by Linear Array HPV Genotyping Method (LA; Roche Molecular Diagnostics, Laval, Quebec, Canada). Hybrid Capture 2 HPV Test (HC2; Qiagen, Gaithersburg, MD), AMPLICOR HPV Test (Amplicor, Roche Molecular Diagnostics, Pleasanton, CA), Real-Time High Risk HPV assay (Abbott Molecular, Abbott Park, IL) and cobas HPV Test (Roche Molecular Diagnostics, Pleasanton, CA) are all tests that detect DNA of 13 or 14 high-risk HPV types. The Aptima HPV assay (Gen-Probe Inc., San Diego, CA) and Pretect HPV-Proofer Test (Proofer; Norchip AS, Klokkarstua,

Norway) detect HPV E6/E7 mRNA of 14 or 5 high-risk HPV types.

There remain several unresolved issues, including developing a preferable screening algorithm to increase the positive predictive value (PPV) of colposcopy referral and how HPV screening performs in China. The HC2 assay is a sensitive test to detect HPV DNA sequences [10], and a negative hrHPV test is associated with the absence of cervical disease [4, 5, 11]. Thus, further triage testing in hrHPV-positive women is needed to identify women with the highest risk for cervical precancerous lesions and cervical cancer [4, 12, 13].

Previous study suggested [14–16] that the risk to develop cancer precursors is genotype related. Carcinomas associated with the hrHPV types, and HPV16, 18 or 45 infection carries a particularly high risk [2, 15, 16]. Whereas the HC2 assay does not distinguish between individual HPV types. APTIMA HPV16 18/45 mRNA assay is a genotyping test for the detection of HPV16, 18 and 45. The clinical significance of HPV16, 18 and 45 mRNA combined with viral load in predicting cervical (pre-) cancer has been studied rarely hitherto. Whether HPV testing can be further optimized by separated detection of the most important HPV types in Chinese women is still unknown.

In the present study, type-specific mRNA probes for HPV16, 18 and 45 were applied to clarify whether women with positive HPV16, 18 and 45 can be benefit for better identification of ≥CIN3. Therefore, the aim of this study is to understand the clinical application of APTIMA HPV16 18/45 mRNA genotyping test by investigating the relationship between oncogenic HPV infection in Chinese population and histological changes in the uterine cervix, which will be benefit for the identification of biomarkers that are suitable for the screening of women who are more prone to ≥CIN3.

Materials and Methods

This study was performed at the Third Hospital of Peking University. All procedures were approved by the local ethics committee.

From November 1, 2013, to January 9, 2014, a total of 394 women who were referred for colposcopy consecutive, and met the eligibility criteria were enrolled.

The eligibility criteria included the age with 20 years old or over, no pregnancy, and no history of pelvic radiation, hysterectomy and previous treatments for cervical cancer or preinvasive lesion, positive hrHPV on the basis of HC-2 assay and atypical squamous cells of undetermined significance or greater on liquid-based cytology.

Upon enrollment, before colposcopy, a single cervical specimen was obtained for HPV testing from all participants using a Cervex broom-type brush (Rovers Medical Devices, Oss, Netherlands), and suspended into PreservCytcollection medium (Hologic Inc., Marlborough, MA), according to the manufacturer's instructions.

Colposcopy and Histology

The colposcopy was completed by two experienced gynecologists and cervical biopsies were performed on the day of patient enrollment according to the standard of care. Two or three diagnostic biopsies were obtained from those subjects with visible lesions under the colposcopy. When lesions were not visible upon satisfactory colposcopic exam, two punch biopsies were performed at 12 and 6 o'clock of transformation zone. Endocervical curettage was performed upon unsatisfactory colposcopic exam.

The 3-tier CIN nomenclature was used for histological diagnosis. Cervical histology was read and reviewed independently by experienced pathologists.

HC2 Assay

HPV screening was conducted using HC2 assay (Digene Corporation, Gaithersburg, MD), a signal amplified hybridization microplate-based assay designated to detect 13 HPV genotypes including hrHPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 through probe cocktails and chemiluminescence. The result is expressed as the relative light unit (RLU) that is calculated as a ratio of the signal from the sample to an average signal from the positive reagent in the kit. The sample is considered as the positive when the RLU/cutoff (CO) ratio is 1.0 or higher.

HPV Genotyping Test

APTIMA HPV16 18/45 mRNA assay (Gen-Probe Inc., San Diego, CA) is designed to detect HPV E6/E7 mRNA from HPV oncogenic types 16, 18 and 45 collected from liquid cervical specimens. The procedures were carried out according to the manufacturer's instructions. Briefly, an aliquot of 1 mL of each PreservCyt sample was transferred to 2.9 mL of buffered detergent solution, and a 400-μL aliquot of the mixture was tested on a fully-automated Panther System.

Statistical Analysis

The clinical performance of HPV tests was assessed on the basis of histological diagnosis, with ≥CIN3 serving as the primary disease endpoint, and the secondary study outcome was ≥CIN2. Sensitivity, specificity, PPV, and negative predictive value (NPV) for ≥CIN2 and ≥CIN3 detection were calculated. And 95 % confidence intervals (CIs) were computed using the binomial method.

Patient backgrounds were compared between ≥CIN3 and <CIN3 groups using Pearson's Chi-Square Tests, respectively.

Differences in oncogenic HPV genotyping between two groups were evaluated using odds risk (OR). Statistical analyses were performed with SPSS software version 13.0 (Chicago, IL, USA). A probability value less than 0.05 was considered as statistically significant difference.

The intensity of HPV infection (i.e., viral load) was assessed by HC2 assay using RLU/CO. Results were stratified into three groups including 1 ≤ RLU/CO < 100, 100 ≤ RLU/CO < 500, and RLU/CO ≥500. In some cases, the 3-level classification was consolidated into 2 levels (1 ≤ RLU/CO <100 and RLU/CO ≥100).

Using HC2 assay results and HPV16 18 and 45 mRNA assay results, HPV infection was defined hierarchically: positive for HPV16 (HPV16+); else positive for HPV18/45 (HPV18/45+; 3 women with HPV16 coinfection were called HPV16+); else negative HPV16, negative HPV18/45, and HC2 positive (HPV16/18/45- coupled with HC2+).

According to the enrollment age, results were stratified into two groups: <30 or ≥30 years old.

Results

Population Backgrounds and Prevalence of Cervical Disease

The age of the participants was 22–68 years old (median age = 35 years).

Of 394 participants, 152 women (38.6 %) had a ≥CIN2 and 55 women (14.0 %) had a ≥CIN3, including 8 carcinomas. The remaining women had no histomorphological abnormalities or CIN 1.

The cases with ≥CIN3 were indentified in all age groups, and the paroxysmal age was come on in 30 to 39 years old (Fig. 1). Only three women were diagnosed as positive by HPV16 18/45 mRNA assay in group ≤24 years old, and in which one woman was diagnosed as CIN3. No ≥CIN3 was diagnosed in group ≤24 years old coupled with HPV16/18/45 mRNA negative.

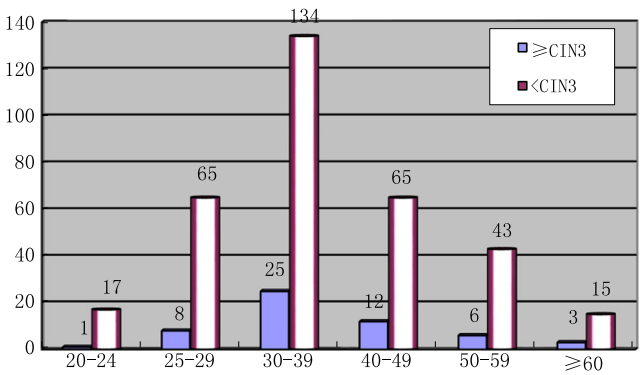

Fig. 1 Distribution of age and cervical disease. ≥CIN3, cervical intraepithelial neoplasia grade 3 or cervical cancer. < CIN3, cervical intraepithelial neoplasia grade 2 or lower

Clinical Performance of APTIMA HPV 16 18/45 mRNA

Among 394 women, 76 women were classified as HPV16+, 55 as HPV18/45+, and 263 as HPV16/18/45- coupled with HC2+. Thus, the prevalence of HPV16/18/45 mRNA in this population was 33.2 % (131/394) (95 % CI, 10.5–17.4 %).

The prevalence of HPV16/18/45 in the older group was 34.7 % (95 % CI, 29.3–40.0 %), which was not significantly higher than that in the younger group (28.6 %, 95 % CI, 19.3–37.9 %) ($P = 0.280$) (Table 1).

As shown in Table 1, the prevalence of HPV16/18/45 was 65.5 % (36/55) (95 % CI, 52.9–78.0 %) among ≥CIN3, which was markedly higher than that group for CIN2 or lower (<CIN 3) (28.0 %, 95 % CI, 23.2–32.8 %) ($P < 0.001$). Of the eight women with cervical cancer, five tested positive by HPV16 18/45 mRNA assay, while the other was negative. Of the three HPV16/18/45 negative-women with cancer, one's cytology result was atypical glandular cells (AGC) and the other two was high-grade squamous intraepithelial lesion (HSIL).

Compared with the group with HPV16/18/45- coupled with HC2+, the OR to identify ≥CIN3 was 6.3 (95 % CI, 3.2–12.3) for HPV16+ and 3.2 (95 % CI, 1.4–7.2) for HPV18/45+. So the prevalence of ≥CIN3 in the group with HPV16+ showed no significant different to the group with HPV18/45+ (Table 2).

The sensitivity for detection of ≥CIN2 of the triage algorithm was 52.0 % (95 % CI, 44.0–59.9 %), while specificities was 78.5 % (95 % CI, 73.3–83.7 %). When using ≥CIN3 as an endpoint, the sensitivity and specificity was 65.5 % (95 % CI, 52.9–78.0 %) and 72.0 % (95 % CI, 67.2–76.8 %), respectively (Table 3).

Table 1 Distribution of cervical disease, age and HPV16 18/45 mRNA assay. Total study population ($n = 394$)

	HPV16/18/45 mRNA positive				
	n1/N1	%	95%CI	*p* value	X^2
Age					
<30	26/91	28.6	(19.3–37.9)	0.280	1.166
≥30	105/303	34.7	(29.3–40.0)		
RLU/CO					
1.00–99.99	28/128	21.9	(14.7–29.0)	0.002	12.485
100–499.99	32/94	34.0	(24.5–43.6)		
≥500.00	71/172	41.3	(33.9–48.6)		
Cervical disease					
≥CIN3	36/55	65.5	(52.9–78.0)	<0.001	29.874
<CIN3	95/339	28.0	(23.2–32.8)		

RLU relative light unit, *CO* cutoff, *CI* confidence interval, *n1* number of test positive disease cases, *N1* total number of disease cases

Table 2 Distribution of HPV genotyping and cervical disease. Total study population ($n = 394$)

	≥CIN3		Odds ratio	95%CI
	n1/N1	%		
HPV16+	25/76	32.9	6.295	(3.226–12.284)
HPV18/45+	11/55	20.0	3.211	(1.430–7.210)
HPV16/18/45- and HC2+	19/263	7.2	1	(reference group)

Table 4 Distribution of HPV16 18/45 mRNA assay and viral load ($n = 394$)

HPV16/18/45 mRNA assay	RLU/CO ≥ 100.00				
	n1/N1	%	95%CI	p value	X^2
Positive	103/131	78.6	(71.6–85.6)	0.001	11.051
Negative	163/263	62.0	(56.1–67.8)		

HPV16 18/45 mRNA Assay Combined with Viral Load and Cervical Disease

We stratified viral loads of women into three groups on a log scale (1 ≤ RLU/CO < 100, 100 ≤ RLU/CO < 500, and RLU/CO ≥500), and calculated the prevalence of HPV16/18/45 mRNA with 95 % CIs. The prevalence of HPV16/18/45 mRNA in group 1 ≤ RLU/CO < 100 was 21.9 % (95 % CI, 14.7–29.0 %), in group 100 ≤ RLU/CO < 500 was 34.0 % (95 % CI, 24.5–43.6 %), and in group RLU/CO ≥500 was 41.3 % (95 % CI, 33.9–48.6 %), respectively. The prevalence of HPV16/18/45 mRNA was increased with the increase of viral load (Table 1) ($P = 0.002$) (Table 1).

We selected a viral load by HC2 of 100 RLU/CO as the cutoff value for discriminating patients with low and high HPV load, and calculated the prevalence of high HPV load in the HPV16/18/45 mRNA positive or negative group with 95 % CIs. The prevalence of high viral load in the HPV16/18/45 positive group was 78.6 % (95 % CI, 71.6–85.6 %), which was markedly higher than that in the HPV16/18/45 negative group (62.0 %, 95 % CI, 56.1–67.8 %) ($P = 0.001$) (Table 4).

We selected the 55 women with ≥CIN3, and calculated the prevalence of high HPV load in the HPV16/18/45 mRNA positive or negative group with 95 % CIs. The prevalence of high viral load in the HPV16/18/45 positive group was 83.3 % (95 % CI, 71.2–95.5 %), which was markedly higher than that in the HPV16/18/45 negative group (47.4 %, 95 % CI, 24.9–69.8 %) ($P = 0.005$) (Table 5).

When the 263 HPV16/18/45 negative-women were selected, and the prevalence of ≥CIN3 between the high HPV load and the low HPV load group with 95 % CIs was calculated. The prevalence of ≥CIN3 in the high viral load group was 5.5 % (95 % CI, 2.0–9.0 %), which was not statistically significant than that in the low HPV load group (10.0 %, 95 % CI, 4.1–15.9 %) ($P = 0.005$) (Table 6).

Discussion

In search of a triage algorithm for hrHPV DNA-positive women, we applied APTIMA mRNA genotyping testing of three hrHPV types in a referral population. The prevalence of HPV16/18/45 mRNA in this population was 33.2 %, and the prevalence of HPV16/18/45 genotypes was 65.5 % (95 % CI = 52.9 to 78.0 %) among ≥CIN3, which was similar with previous reports [17–19]. It indicated that HPV16/18/45 genotypes were the most common HPV types for ≥CIN3 in Chinese women.

Compared with the group with negative HPV16/18/45 and positive HC2, the OR to identify ≥CIN3 was 6.3 (95 % CI, 3.2–12.3 %) for HPV16+ and 3.2 (95 % CI, 1.4–7.2) for HPV18/45+. It indicated that presence of HPV16/18/45 strongly increased the risk of ≥CIN3. HPV screening that distinguishes HPV16, 18 and 45 from other oncogenic HPV types may identify women at the greatest risk of ≥CIN3, and the HPV16 18/45 mRNA assay would be available for the further triaged of the HC2 positive cases in case of the HPV primary screening performs in China. The sensitivity and specificity for detection of ≥CIN2 and ≥CIN3 were similar to those of HPV16/18 DNA genotyping in other study [19]. Therefore HPV16/18/45 mRNA -positive women should be referred to colposcopy immediately.

Our present finding showed that only one woman was diagnosed as CIN3 in group ≤24 years old (1/18), she was positive by HPV16 18/45 mRNA assay. Whether or not the HPV16 18/45 mRNA assay can be used in the primary

Table 3 Clinical performance of HPV16 18/45 mRNA assay to detect ≥CIN2 or ≥CIN3 in HC2-positive women. Total study population ($n = 394$)

	n1/N1	Sensitivity	(95 % CI)	n2/N2	Specificity	(95 % CI)	PPV	(95 % CI)	NPV	(95 % CI)
≥CIN2	79/152	52.0 %	44.0–59.9 %	190/242	78.5 %	73.3–83.7 %	60.3 %	51.9–68.7 %	72.2 %	66.8–77.7 %
≥CIN3	36/55	65.5 %	52.9–78.0 %	244/339	72.0 %	67.2–76.8 %	27.5 %	19.8–35.1 %	92.8 %	89.6–95.9 %

CIN cervical intraepithelial neoplasia, *CI* confidence interval, *PPV* positive predictive value, *NPV* negative predictive value, *n1* number of test positive disease cases, *N1* total number of disease cases, *n2* number of test negative non-disease cases, *N2* total number of non-disease cases

Table 5 Distribution of HPV genotyping combined with cervical disease and viral load ($n = 55$)

	RLU/CO ≥ 100				
≥CIN3	n1/N1	%	95%CI	*p* value	X^2
HPV 16/18/45+	30/36	83.3	(71.2–95.5)	0.005	7.798
HPV 16/18/45−	9/19	47.4	(24.9–69.8)		

cervical screening at 20–24 years old women, the data was limited in number, further research is needed.

Some of studies [20, 21] used HC2 value as Viral load. Our present finding showed that viral loads were largely variable. As a result of its broad distribution, viral load was not support to use as a disease biomarker [20, 21]. Genotyping may therefore improve risk stratification of women with HPV in cervical screening program [14, 22].

We found that the prevalence of HPV16/18/45 mRNA was increased with the increase of viral load (Table 1), and when a woman was HPV16/18/45 mRNA positive, she always with high viral load (Table 4). Furthermore, we found that ≥CIN3 coupled with positive HPV16/18/45 was associated significantly with the prevalence of high viral load than the ≥CIN3 group coupled with negative HPV16/18/45. It indicated that, different HPV types may have diverse oncogenic mechanisms, which needs to be explored. The HPV16 and its viral load have been reported to be associated with cervical cancer and cancer precursors, can be used as a potential biomarker to predict the future cervical lesion progression [23–26]. Hence, HPV 16 18/45 E6/E7 mRNA combined with high viral load may be used as a disease biomarker, which also needs to be further explored in Chinese cohorts.

HPV 16, 18 and 45 account for approximately 70–80 % of HPV types encountered worldwide [2, 16, 17]. Thus testing HPV16 18/45 mRNA alone is not sufficient for monitoring tumor. Management of the HPV16/18/45 negative cases requires further research. Some studies suggest that the hrHPV viral load is recommended to triage the hrHPV-positive women [27, 28]. Our data showed that in cases with negative HPV 16 18/45 mRNA, high viral load did not predict sever cervical lesions (Table 6). As a result, viral load was not suitable for the further triaged of the HPV16/18/45 negative women. In the present study, only three cancer cases was HPV16/18/45 negative, their cytology results were HSIL or AGC, it is shown that HPV 16 18/45 mRNA combination with cytology as triage strategies would be enough safety.

Table 6 Distribution of HPV genotyping combined with viral load and cervical disease ($n = 263$)

HPV16/18/45−	≥CIN3				
	n1/N1	%	95%CI	*p* value	*X*2
RLU/CO < 100	10/100	10.0	(4.1–15.9)	0.173	1.855
RLU/CO ≥ 100	9/163	5.5	(2.0–9.0)		
Total	19/263	7.2			

In summary, the APTIMA assay targets the E6/E7 genes implicated in carcinogenesis. HPV16 18/45 mRNA genotype test would have a good value in cervical cancer screening. The APTIMA HPV 16 18/45 mRNA assay could be used for the future triage in HPV primary screening programs, or perhaps could be used as a stand-alone primary screening test. Screening programs based on the combination of Pap test and HPV DNA test improve the detection of cervical (pre-) cancer, and also have limitations. The sensitivity of the combinatorial test from both is higher than that of the test alone although the clinical specificity and PPV for ≥CIN3 are not high enough. A screening program with combinatorial test from two assays with high specificity could reduce the amount of colposcopies and the unavoidable cost of overtreatment. For example, APTIMA HPV 16 18/45 mRNA assay can be used as the primary screening test, and women with positive HPV16/18/45 should be referred to colposcopy immediately and closer follow-up, but others should be further triaged by another test with high PPV for ≥CIN3. The clinical performance should be further explored.

However, there are few limitations in this study. The HC2 system (Digene) for hrHPV detection is a signal-amplified hybridization antibody capture assay, and the RLU/CO represents a semiquantitative value for the cumulative viral burden of one or more of the 13 oncogenic HPV types. The cumulative HPV viral load may represents the sum of multiple infections without the possibility to determine the real contribution of a single HPV-genotype replication. On the other hand, the number of cells of the sample is not normalization. The HC2 assay therefore does not play to reliably determine HPV DNA levels.

Whereas no one normalized quantitative measurement assay that satisfactorily determine HPV DNA levels, such as Real-time PCR (QPCR), was allowed to clinical performance. HC2 is still a reliable clinical test for oncogenic HPV DNA detection, and demonstrated good interlaboratory agreement [29].

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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