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Evaluation of the Human Papillomavirus mRNA Test for the detection of cervical lesions in Japan

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Summary

Aims. For the screening of cervical abnormalities, human papillomavirus (HPV) DNA testing is widely used along with Papanicolaou (Pap) testing. Although the sensitivity of the HPV DNA testing is good, its specificity is relatively low. In the present study, the authors evaluated the use of the Gen-Probe APTIMA HPV Assay for the detection of HPV mRNA and compared it with HPV DNA testing. *Materials and Methods.* Liquid cervical Pap specimens collected from 410 women were assessed using the APTIMA test, the Qiagen Hybrid Capture 2 HPV DNA (HC2) Test, and the AMPLICOR HPV Test. *Results.* The sensitivity and specificity for the detection of high-risk HPV were 85.6% and 99.2% for the APTIMA test, 94.1% and 98.4% for the HC2 test, and 90.2% and 95.7% for the AMPLICOR test, respectively. As the severity of the cervical lesion progressed, the positive rate of the three tests indicated a similar increase. The clinical sensitivity and specificity for the detection of squamous intraepithelial lesion (SIL) were 91.2% and 84.2% for the APTIMA test, 94.5% and 80.4% for the HC2 test, and 87.9% and 78.2% for the AMPLICOR test, respectively. *Conclusion.* The APTIMA is sensitive and specific for the detection of high-risk HPV. In the specimens with SIL, the APTIMA test is more specific than the HC2 and the AMPLICOR tests. This indicates that the APTIMA test may improve patient management and reduce the cost of screening.

Key words: Cytology; Human papillomavirus; mRNA; Specificity; Squamous intraepithelial lesion.

Introduction

Uterine cervical cancer is the most common gynecological cancer. This disease affects 15 women per 100,000 women annually in Japan, and its incidence has recently been increasing. Almost all cases of cervical cancer and its precursor lesion, cervical intraepithelial neoplasia (CIN), are caused by the human papillomavirus (HPV), and prolonged infection with high-risk HPV (HR-HPV) is particularly likely to cause cervical cancer [1, 2]. Most women experience one or more infections by HPV in their lifetime; however, more than 90% of these infections are transient because the human immune system can eradicate the HPV within two years [3]. However, in a small percentage of HPV infections, the virus persists in the cervical epithelium and is integrated into the host DNA, leading to the formation of a cervical lesion.

HPV is a DNA virus, and the viral genome consists of 7900 bp that encode eight open reading frames (ORFs). The E6 and E7 genes in these ORFs have a great influence on the formation of cervical lesions. The products of these two genes induce uncontrollable cell proliferation by inactivation of p53 and pRb [4, 5]. The cervical epithelial cells express E6/E7 mRNA at a constant high level due to prolonged HPV infection and integration of HPV into their DNA.

Brush cytology has been widely used for the detection of cervical lesions worldwide. The HPV DNA test has recently been used in cases where an abnormality is detected by brush cytology. In Western countries, the HPV DNA test has recently been widely adopted for cervical examination, and because the HPV DNA test is more sensitive than brush cytology, the simultaneous use of both tests is recommended for detecting early cervical lesions [6-8].

A study from the Netherlands showed that screening with the HPV test prior to cytology also improves the effectiveness and decreases the costs associated with cervical cancer examination [9].

However, the DNA HPV test has a low specificity and does not entirely reflect the progression of the cervical lesion since it detects transient HPV infection [8, 10].

The use of HPV tests that detect HPV mRNA has recently been increasing. The APTIMA HPV Assay, one such HPV test, has been approved by Food and Drug Administration (FDA) and is also currently available for sale in more than ten European Union countries.

In the present study, the authors aimed to evaluate the clinical performance of the APTIMA test for the detection of cervical lesions in Japan, and compared it with the HPV DNA tests that are already in use in Japan.

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Table 1. — *Comparison of the APTIMA, HC2, and AMPLICOR tests with regard to the high-risk HPV status.*

		Cases of high-risk HPV		
		Positive	Negative	Total
APTIMA	Positive	131	2	133
	Negative	22	255	277
	Total	153	257	
HC2	Positive	144	4	148
	Negative	9	253	262
	Total	153	257	
AMPLICOR	Positive	138	11	149
	Negative	15	246	261
	Total	153	257	

APTIMA: Gen-Probe APTIMA HPV Assay;
HC2: Hybrid Capture 2 HPV DNA Test;
AMPLICOR: AMPLICOR HPV Test; HPV: human papillomavirus.

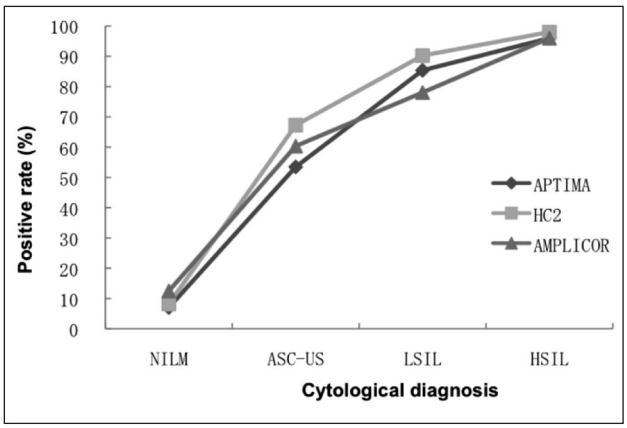

Figure 1. — The positive rate of the APTIMA, HC2, and AMPLICOR tests according to the cytological diagnosis is shown. As the lesion progressed from a grading of negative for intraepithelial lesion or malignancy (NILM) to high-grade intraepithelial lesion (HSIL), the positive rate of all three tests showed a similar increase. LSIL: low-grade squamous intraepithelial lesion; ASC-US: atypical squamous cells of unknown significance; APTIMA, Gen-Probe APTIMA HPV Assay; HC2: Qiagen Hybrid Capture 2 HPV DNA Test; AMPLICOR: AMPLICOR HPV Test.

Materials and Methods

Population and sampling

A total of 410 cervical specimens were acquired from four hospitals in Japan (Kosei Chuo General Hospital, Kamata General Hospital, Sanno Medical Center, and Kosugi Clinic) between November 2011 and April 2012. Specimens were obtained from women who underwent a cervical cytology examination for the following reasons: (1) cervical cancer screening; (2) cervical cytology testing as cancer was suspected; (3) need for a re-examination based on the result of a previous cytology examination; and (4) undergoing treatment for cervical cancer. The mean age of the women was 39 ± 9.3 years (range, 20–76). In all the cases, cervical cytological examinations were performed using the Cervex-Brush and the cervical samples were preserved in PreservCyt solution. The cytologic specimens were prepared from this solution according to the liquid-based cytology (LBC) methods, and HPV tests were simultaneously performed. Woman who provided written informed consent were enrolled. The Ethics committee of Tokyo Kosei Chuo General Hospital approved all protocols.

Cytology

Cytological diagnosis was made by cytotechnologists and cytopathologists according to the Bethesda system. The potential diagnoses included negative for intraepithelial lesion or malignancy (NILM), low-grade squamous intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), atypical squamous cells of unknown significance (ASC-US), atypical squamous cells–cannot exclude HSIL (ASC-H), or squamous cell carcinoma (SCC).

HPV mRNA test

The Gen-Probe APTIMA HPV Assay was used for HPV mRNA testing. A one-ml sample from the preserved solution was placed in a test tube containing a buffer solution to lyse the cells and extract their mRNA. The test tube was then loaded onto a fully automated TIGRIS DTS system and the solution was analyzed according to the manufacturer's instructions. The APTIMA test can detect the E6/E7 mRNA of 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

HPV DNA test

The Hybrid Capture 2 HPV DNA Test (HC2) and the AMPLICOR HPV Test, both of which were already under license in Japan at the start of this study, were selected for HPV DNA testing. These two tests detect 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Aliquots of four ml and 250 µl from the preserved solution were used for HC2 and AMPLICOR testing, respectively.

Evaluation of the HPV tests

Three cases using the APTIMA, HC2, and AMPLICOR tests were conducted. The first case indicated a unanimous HR-HPV positive result. The second case indicated a unanimous HR-HPV negative result. However, deviations were noted during the testing of the third case, and therefore, a Linear Array HPV Genotyping Test (LA) was used. The sample in which HR-HPV was detected on LA testing was considered to be HR-HPV positive.

Statistical analysis

The authors calculated the sensitivity, specificity, and positive and negative predictive values (PPV/NPV) by using two × two tables, and the results are described with 95% confidence intervals. The probabilities were compared using McNemar's test. Data analysis was performed using the SPSS software.

Results

Of the 410 women who provided cervical specimens, 153 cases were found to be HR-HPV positive, whereas 257 were found to be HR-HPV negative (Table 1). Of the 153 HR-HPV-positive cases, 121 were found to be positive by all three tests and the other 32 HR-HPV cases were diagnosed by LA HPV typing. In the HR-HPV-positive group, 131 cases (85.6%) were found to be HR-HPV positive and 22 (14.4%) were found to be HR-HPV negative by APTIMA testing. Of the 22 cases that were HR-HPV positive but were negative by APTIMA testing, the cytological diagnosis was NILM in 12, ASCUS in eight, and LSIL in two

Table 2. — *Clinical sensitivity and specificity of the APTIMA, HC2, and AMPLICOR tests for the detection of high-risk HPV.*

	APTIMA	HC2	AMPLICOR
Sensitivity (95% CI)	85.6	94.1	90.2
	(79.0 - 90.8)	(89.1 - 97.2)	(84.4 - 94.4)
p-value*		< 0.01	0.2
Specificity (95% CI)	99.2	98.4	95.7
	(97.2 - 99.9)	(96.1 - 99.6)	(92.5 - 97.8)
p-value*		0.62	< 0.05
PPV	98.5	97.3	92.6
NPV	92.1	96.6	94.3

APTIMA: Gen-Probe APTIMA HPV Assay;
HC2: Qiagen Hybrid Capture 2 HPV DNA Test;
AMPLICOR: AMPLICOR HPV Test; CI: confidence interval;
HPV: human papillomavirus; PPV: positive predictive values;
NPV: negative predictive values.
* McNemar's test results when comparing to APTIMA test.

Table 3. — *Clinical sensitivity and specificity of the APTIMA, HC2, and AMPLICOR tests for the detection of high-risk HPV in cases with a cytological diagnosis of SIL.*

	APTIMA	HC2	AMPLICOR
Sensitivity (95% CI)	91.2	94.5	87.9
	(83.4 - 96.1)	(87.6 - 98.2)	(79.4 - 93.8)
p-value*		0.25	0.37
Specificity (95% CI)	84.2	80.4	78.2
	(79.7 - 88.1)	(75.6 - 84.7)	(73.3 - 82.7)
p-value*		< 0.01	< 0.05
PPV	62.4	58.1	53.7
NPV	97.1	80.4	95.8

APTIMA: Gen-Probe APTIMA HPV Assay;
HC2: Qiagen Hybrid Capture 2 HPV DNA Test;
AMPLICOR: AMPLICOR HPV Test; CI: confidence interval;
HPV: human papillomavirus; PPV: positive predictive values;
NPV: negative predictive values; SIL: squamous intraepithelial lesion.
* McNemar's test results when comparing to APTIMA test.

cases. Of the HR-HPV-negative cases, 255 (99.2%) were found to be negative and two (0.8%) were found to be positive by APTIMA testing. Of the two cases that were HR-HPV negative but positive by APTIMA testing, the cytological diagnosis was NILM in one and ASCUS in the other case.

Of the HR-HPV-positive cases, 144 (94.1%) were found to be positive and nine (5.8%) were found to be negative by HC2 testing. Of the nine cases that were HR-HPV positive but were negative by HC2 testing, the cytological diagnosis was NILM in eight and ASCUS in one case. Of the HR-HPV-negative cases, 253 (98.4%) were found to be negative and four were found to be positive on HC2 testing. Of the four cases that were HR-HPV negative and were also negative on HC2 testing, the cytological diagnosis was ASCUS in two, ASC-H in one, and HSIL in one case. Of the HR-HPV-positive cases, 138 (90.2%) were found to be positive and 15 (9.8%) were found to be negative by AMPLICOR testing. Of the 15 cases that were HR-HPV positive but negative on AMPLICOR testing, the cytological diagnosis was NILM in four, ASCUS in five, LSIL in five, and HSIL in one case. Of the cases that were HR-HPV negative, 246 (95.7%) were found to be negative and 11 (4.3%) were found to be positive on AMPLICOR testing. Of the 11 cases that were HR-HPV negative but were positive on AMPLICOR testing, the cytological diagnosis was NILM in seven, ASCUS in two, ASC-H in one, and HSIL in one case.

Subsequently, the authors examined the HR-HPV positive rate of all the three tests according to the cytological diagnosis. The positive rate of the three tests showed a similar pattern of increase as the lesion progressed from NILM to HSIL (Figure 1). The sensitivity for HR-HPV was 85.6% for the APTIMA test and 94.1% for the HC2 test (Table 2). The sensitivity of the HC2 test was significantly higher than that of the APTIMA test ($p < 0.01$); however, no significant difference in specificity was noted between these tests. The sensitivity for HR-HPV was 85.6% for the APTIMA test and 90.2% for the AMPLICOR test; however, no difference in sensitivity was noted between these tests (Table 2). In contrast, the specificity for the APTIMA test (99.2%) was significantly greater than that of the AMPLICOR test (95.7%) ($p < 0.05$).

Table 4. — *Clinical sensitivity and specificity of the APTIMA, HC2 and AMPLICOR tests for the detection of high-risk HPV in cases with a cytological diagnosis of HSIL*

	APTIMA	HC2	AMPLICOR
Sensitivity (95% CI)	96.0	98.0	96.0
	(86.3 - 99.5)	(89.4 - 99.9)	(86.3 - 99.5)
p-value*		1	0.48
Specificity (95% CI)	76.3	72.4	71.2
	(74.5 - 80.6)	(67.4 - 76.9)	(66.8 - 76.4)
p-value*		< 0.01	< 0.05
PPV	36.1	33.1	32.2
NPV	99.3	99.6	99.2

APTIMA: Gen-Probe APTIMA HPV Assay;
HC2: Qiagen Hybrid Capture 2 HPV DNA Test;
AMPLICOR: AMPLICOR HPV Test; CI: confidence interval;
HPV: human papillomavirus; PPV: positive predictive values;
NPV: negative predictive values; HSIL: high-grade intraepithelial lesion.
* McNemar's test results when comparing to APTIMA test.

The authors then compared the results of the three tests in the 91 cases with a cytological diagnosis of SIL (comprising LSIL and HSIL). In these cases, the sensitivity was 91.2% for the APTIMA test and 94.5% for the HC2 test; however no significant difference was noted in the sensitivity between these tests (Table 3). In contrast, the specificity of the APTIMA test (84.2%) was significantly greater than that of the HC2 test (80.4%) ($p < 0.01$).

Although no significant difference was noted in the sensitivity between the APTIMA and the AMPLICOR tests (Table 3), the specificity of the APTIMA test (84.2%) was greater than that of the AMPLICOR test (78.2%) ($p < 0.05$).

Among the 50 cases diagnosed as HSIL, the sensitivity was 96.0% for the APTIMA test and 98.0% for the HC2 test; however, no significant difference in the sensitivity was noted between the tests (Table 4). In contrast, the specificity was 76.3% for the APTIMA test and 72.4% for the HC2 test ($p < 0.01$). Although no significant difference in sensitivity was noted between the two tests (Table 4), the specificity of the APTIMA test (76.3%) was significantly greater than that of the AMPLICOR test (71.2%) ($p < 0.05$).

Discussion

Uterine cervical cancer is an important cancer as almost all types of cervical cancer are caused by an HR-HPV infection [11]. Because many adult women have been infected with HR-HPV at least once during their lifetime, HPV testing is used worldwide for cervical cancer examination. DNA and mRNA tests are currently available for HPV detection [12]. Although the HPV DNA test has been widely used to detect the presence of the HPV genome, this method also detects the presence of a transient HPV infection that may never cause a cervical lesion, resulting in a relatively low specificity [10]. The HPV mRNA test has recently attracted attention as a novel method to replace the HPV DNA test. Because the development of cervical lesions requires high levels of expression of the HPV-derived E6/E7 genes [4], the HPV mRNA test is believed to more accurately reflect the onset and progression of cervical lesions. In the present study, the APTIMA test showed high sensitivity (85.6%) and specificity (94.1%) for HR-HPV detection in cases with cervical lesions. Previous studies comparing the APTIMA and HPV DNA tests showed that the APTIMA test had similar sensitivity but better specificity as compared to the HPV DNA tests [13-15]. However, in the present study, the APTIMA test was more specific than the AMPLICOR test, but the sensitivity of the APTIMA test was significantly lower than that of the HC2 test. Moreover, the APTIMA test did not indicate a superior sensitivity or specificity in any of the HPV-infected specimens.

HPV 66 is one of many HPV types that cause cervical cancer [16]. As HPV 66 can only be detected by the APTIMA test, the authors expected the sensitivity of the APTIMA test to be higher than that of the HC2 and AMPLICOR tests. However, both the APTIMA and HC2 tests showed positive results in all the five cases wherein HPV 66 was detected by LA HPV typing. The authors believe that this one of the reasons why the APTIMA test was not found to be superior to the HC2 test in the present study. This unexpected detection of HPV 66 by the HC2 test may represent a type of cross reaction, which is a phenomenon that has been reported in prior studies [15, 17].

mRNA, the target of the APTIMA test, is less stable than DNA, and this instability is believed to cause a decline in sensitivity. However, in the present study, the authors used a proteolytic enzyme in the preserved solution (the LBC method), which has been shown to preserve RNA in a stable form for two to five weeks. Therefore, the authors believe that mRNA instability did not significantly affect the present results [18-20].

The cases diagnosed with SIL (including LSIL or HSIL) by cytological testing showed equal sensitivity and higher specificity compared to HPV DNA tests, which is consistent with a study on APTIMA tests in Western countries [15, 19, 21, 22]. Although the authors did not perform histological examination in the current study, some studies has shown that the HPV E6/E7 mRNA test using the RT-PCR method is more specific than the HPV DNA test for the diagnosis of CIN [20, 23]. HPV DNA tests may be less specific than HPV mRNA tests because, as stated above, DNA tests can detect transient HPV infection and also exhibit cross reactivity with certain low-risk HPV types [24]. Since HR-HPV cases detected by the HPV mRNA test have a greater tendency to progress to CIN over a long period than those detected by HPV DNA testing [25], the higher specificity of the APTIMA test may only become apparent during the follow-up of the cases in the present study.

The combined use of cytological examination and the HPV test is currently recommended for the prevention and early detection of cervical lesions, and this recommendation is being increasingly followed in Japan. However, only 5% of the patients who are infected with HR-HPV eventually develop cervical cancer [26]. Therefore, a more specific detection method is desirable in order to reduce examination costs. Since the APTIMA test has a higher specificity compared with the HPV DNA tests, the authors believe that this test could replace the HPV DNA tests in Japan.

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