RESEARCH COMMUNICATION

Significance of Human Telomerase RNA Gene Amplification Detection for Cervical Cancer Screening

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Abstract

Aim: Liquid-based cytology is the most often used method for cervical cancer screening, but it is relatively insensitive and frequently gives equivocal results. Used as a complementary procedure, the high-risk human papillomavirus (HPV) DNA test is highly sensitive but not very specific. The human telomerase RNA gene (TERC) is the most often amplified oncogene that is observed in cervical precancerous lesions. We assessed genomic amplification of TERC in liquid-based cytological specimens to explore the optimal strategy of using this for cervical cancer screening. Methods: Six hundred and seventy-one residual cytological specimens were obtained from outpatients aged 25 to 64 years. The specimens were evaluated by the Digene Hybrid Capture 2 (HC2) HPV DNA test and fluorescence *in situ* hybridization (FISH) with a chromosome probe to TERC (3q26). Colposcopic examination and histological evaluation were performed where indicated. Results: The TERC positive rate was higher in the CIN2+ (CIN2, CIN3 and SCC) group than in the normal and CIN 1 groups (90.0% vs. 10.4%, $p < 0.01$). In comparison with the HC2 HPV DNA test, the TERC amplification test had lower sensitivity but higher specificity (90.0% vs. 100.0%, 89.6% vs. 44.0%, respectively). TERC amplification test used in conjunction with the HC2 HPV DNA test showed a combination of 90.0% sensitivity and 92.2% specificity. Conclusion: The TERC amplification test can be used to diagnose cervical precancerous lesions. TERC and HPV DNA co-testing shows an optimal combination of sensitivity and specificity for cervical cancer screening.

Keywords: Cervical neoplasms - human telomerase RNA gene - FISH - HPV - hybrid capture 2

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Introduction

Cervical cancer is the second most common malignant tumor in women worldwide, and is caused primarily by persistent infection with the high-risk human papillomavirus (HPV). Most of the HPV infections are self-limiting and can be eradicated, whereas a minority of the infections become integrated ones. The HPV infection is known to be necessary but not sufficient to progress to high-grade cervical lesions and cervical cancer. The contrast between the high rate of HPV infection and the low rate of cervical cancer morbidity suggests that additional genetic events occur during malignant progression (Zhang et al., 2002). The oncogene amplification is frequently observed in the cervical precancerous lesion according to the comparative genomic hybridization (CGH) study results (Heselmeyer et al., 1997; Kirchhoff et al., 1999) and is a fairly early event in cervical carcinogenesis.

Nowadays, liquid-based cytology and Hybrid Capture 2 (HC2) HPV DNA test have become the two most commonly used methods for cervical cancer screening. A single cytological examination is relatively insensitive and easily gives controversy interpretations. Used as a complementary procedure, HC2 HPV DNA test provides extremely high sensitivity and negative predictive value (NPV) (Solomon et al., 2001; Castle et al., 2002). However, low specificity and low positive predictive value (PPV) of the HC2 HPV DNA test limit its clinical application. In clinical practice, most of the low-grade lesions are likely to regress spontaneously and can be closely monitored in defined intervals, whereas high-grade lesions require immediate surgical treatment (Cox et al., 2003). This has prompted efforts to discover other biomarkers with the potential of high specificity as well as excellent sensitivity for the detection of high-grade lesion and cervical cancer.

We reviewed relevant literature and selected the human telomerase RNA gene TERC (3q26), the most commonly observed amplified oncogene in cervical precancerous lesion, as the biomarker for the cervical lesion diagnoses (Heselmeyer et al., 1997; Kirchhoff et al., 1999; Sokolova et al., 2007; Policht et al., 2010). Fluorescence in situ hybridization (FISH) was conducted to check the TERC amplification in the residual liquid-based cytological specimens, and the high-risk HPV infections were tested by the HC2 method. Relationship

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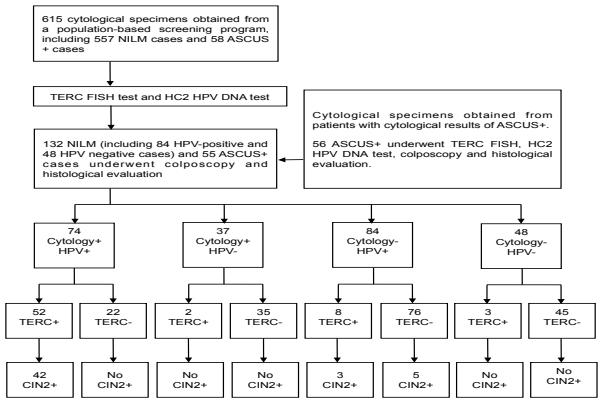

Figure 1. Flowchart of Enrollment and Outcomes of Participants in the Study. Screening positive threshold: Cytology+ determined as ASCUS or higher. HPV+ determined as RLU/CO ≥ 1.0 with a HC2 HPV DNA test. TERC+ determined as ≥ 5% aberrant TERC cells of the cells observed

among TERC amplifications, HPV infections and the clinicopathologic parameters of the cervical lesions were evaluated. The diagnostic performance of various combinations of cytology analysis, HC2 HPV DNA test and TERC amplification test was analyzed for an optimal design of screening strategies.

Materials and Methods

Cytological specimens

PreservCyt (Cytyc) cytological specimens from 671 outpatients (aged 25 to 64 years) seen at Qilu Hospital of Shandong University, Weifang People's Hospital, and the Affiliated Hospital of Medical College of Qingdao University, between August 2010 and October 2011 were obtained, including 557 cases of negative for intraepithelial lesion or malignancy (NILM), 52 cases of atypical squamous cells of undetermined significance (ASCUS), 21 cases of low-grade squamous intraepithelial lesion (LSIL), 14 cases of atypical squamous cells that cannot be excluded for high-grade squamous intraepithelial lesion (ASC-H), 24 cases of high-grade squamous intraepithelial lesion (HSIL) and 3 cases of squamous cell carcinoma (SCC). Patients who have histologically confirmed cervical intraepithelial neoplasia (CIN), and had undergone colposcopic examinations or treatment for cervical lesions were excluded from the study. All of the NILM cases and 58 cytologically abnormal cases (ASCUS or worse; ASCUS+) were obtained from an opportunistic screening program between August 2010 and February 2011. To facilitate statistical analysis, another 56 ASCUS+ cases were recruited from February 2011 to October 2011 (see flow chart in Figure 1) (Chen et al., 2012). The study had been approved by the ethics committee of Qilu Hospital, Shandong University and was performed in accordance with the ethical standards. All of the specimens were used for the present study with the informed consent of the patients. The specimens were analyzed by liquid-based cytology and classified according to the 2001 Bethesda System (Solomon et al., 2004). Consensus was reached by two cytopathologists if there was controversial interpretation. After cytological analysis, residual cytological specimens were used for the TERC amplification test and the HC2 HPV DNA test.

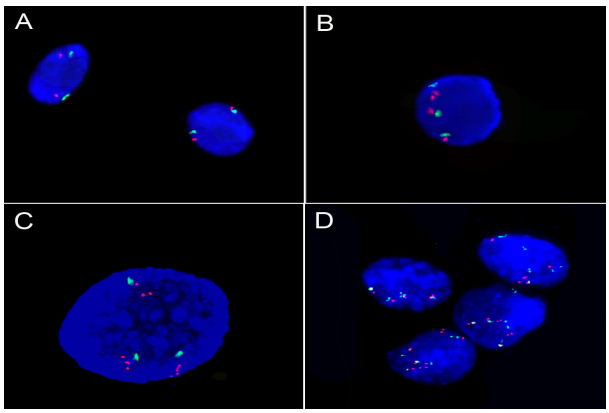

Figure 2. Representative Images of TERC-CEP3 Signals Observed in Cervical Epithelial Cells. (A) Normal epithelial cells revealed a signal pattern of 2-2 (patterns are described in the order TERC-CEP3). (B) Abnormal signal pattern of 4-3. (C) Abnormal signal pattern of 11-3. (D) Abnormal signal pattern cells lay next to each other

FISH analysis and signal enumeration

The cervical cancer-specific FISH probe panel consisted dual-color probes TERC (labeled with spectrum red) and centromere 3 (CEP3, labeled with green as internal control). The probes were provided by GP Medical Technologies (Beijing, China). Five to ten milliliter cell preserved liquid was centrifuged to collect cells. The collected cells were incubated with collagen B at 37 °C for 20 minutes, with deionized water at 37 °C for 30 minutes and were twice fixed in methanol-acetic acid (3:1) for 10 minutes. The pretreated cells were transferred to the slide by a dropper and dried at 56 °C for 30 minutes. The slides were washed twice in 2×SSC for 5 minutes, treated with 0.1 M HCl for 10 minutes, digested with 0.02 mg/ml pepsin/0.01 M HCl at 37 °C for 10 minutes, fixed in 2.5% formaldehyde/PBS for 10 minutes, dehydrated in an ethanol series and dried in the air. The slides and probes were denatured simultaneously at 75 °C for 5 minutes followed by hybridization in a wet box at 42°C for 16 hours. The cover slips were removed, and the slides were washed twice in 0.3% NP-40/0.4×SSC at 67 °C, in 0.1% NP-40/2×SSC for 30 seconds, and in 70% ethanol for 3 minutes. The slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) for 10 to 20 minutes, and images were acquired using Olympus BX51 fluorescence microscope (Tokyo, Japan) that was connected to a ProgRes Mfcool JENOPTIK camera (Jena, Germany). The signals were evaluated by screening the whole slide with a 100× objective through a DAPI filter to determine the cell areas. We enumerated TERC signals through a red filter and CEP3 signals through a green filter. We evaluated at least 100 nuclei and calculated the proportion of aberrant cells for each specimen (Tu et al., 2009). Normal cervical epithelial cells contain 2 TERC signals and 2 CEP3 signals in a nucleus. Cells were considered abnormal if more than 3 TERC signals were detected in a nucleus (Figure 2). The pathologists who enumerated the signals were unaware of the cytological and histological results. A consensus diagnosis was made by two pathologists in cases of controversial enumerations.

Table 1. HPV Positive Rates and TERC Positive Rates for Various Cytological Diagnoses

Cytological Diagnosis	HPV Positive Cases % (Positive/total)	P	TERC Positive Cases % (Positive/total)	P
NILM	24.1 (134/557)		2.7 (15/557)	
ASC-US	50.0 (26/52)		19.2 (10/52)	
LSIL	71.4 (15/21)		52.4 (11/21)	
ASC-H	71.4 (10/14)		64.3 (9/14)	
HSIL	91.7 (22/24)		91.7 (22/24)	
SCC	100.0 (3/3)		100.0 (3/3)	
NILM vs. ASC/LSIL†		< 0.01*		< 0.01*
ASC/LSIL vs. HSIL/SCC		< 0.01*		< 0.01*
LSIL/lower vs. HSIL/higher		< 0.01*		< 0.01*

†ASC includes ASC-US and ASC-H; *P < 0.05: statistically significant difference between 2 categories

Table 2. HPV Positive Rates and TERC Positive Rates for Various Histological Diagnoses

Histological Diagnosis	HPV Positive Cases % (Positive/total)	P	TERC Positive Cases % (Positive/total)	P
Normal	51.2 (84/164)		9.2 (15/164)	
CIN1†	82.8 (24/29)		17.2 (5/29)	
CIN2	100.0 (21/21)		76.2 (16/21)	
CIN3	100.0 (22/22)		100.0 (22/22)	
SCC	100.0 (7/7)		100.0 (7/7)	
Normal vs. CIN1		< 0.01*		> 0.05
CIN1 vs. CIN2+		< 0.01*		< 0.01*
Normal/CIN1 vs. CIN2+		< 0.01*		< 0.01*

†CIN1 includes lesions previously classified as mild dysplasia, koilocytotic atypia, koilocytosis and flat condyloma; *P < 0.05: statistically significant difference between 2 categories

In the present study, the cut-off value determined for the TERC amplification test was 5% or more aberrant TERC cells (Chen et al., 2012).

High-risk HPV DNA test

Cervical cells were collected and used for the high-risk HPV DNA detection with the Digene HC2 method (Qiagen, Gaithersburg, MD) according to the instruction. High-risk HC2 HPV DNA test result with relative light units over cut-off (RLU/CO) value of more than 1.0 was regarded as positive.

Colposcopic examination and histological evaluation

Patients were recommended to undergo colposcopic examinations within 4 months if one of the three tests (cytology analysis, HC2 HPV DNA test or TERC amplification test) showed positive result. Colposcopy-directed biopsy and histological evaluation were then performed. A cervical biopsy diagnosis of CIN2+ was considered as high-grade lesion. One hundred and ninety-eight cases with positive results undergone colposcopic examinations and were histologically confirmed. As a control, a consecutive sample of 45 cases from the 420 cases with negative results was invited to undergo colposcopic examination and histological evaluation.

Statistical analysis

A chi-squared test was used for analysis of the categorical data. Statistical significance was set at P value less than 0.05. Youden's index (Y = sensitivity + specificity - 1) was used to evaluate the combined sensitivity and specificity of the diagnostic methods.

Results

TERC amplification rate in association with cytopathologic and histopathologic evaluations

The numbers of cases with HPV infections and with TERC amplifications by cytological diagnoses are shown in Table 1. One hundred and thirty-two NILM cases underwent colposcopic examinations and histological evaluations, and 84 HPV+ cases and 11 TERC+ cases were observed. No CIN2+ lesion was found in all of the HPV-cases. Of the 76 HPV+/ TERC- cases and the 8 HPV+/ TERC+ cases, 5 CIN2+ cases (6.6%) and 3 CIN2+ cases (37.5%) were detected, respectively. In the 50 ASCUS cases that were histologically confirmed, no CIN2+ lesion was detected of the 15 HPV+/TERC- cases, whereas 4 CIN2+ were detected of the 9 HPV+/TERC+ cases. Of the 21 LSIL cases, 5 HPV+/TERC- cases showed no CIN2+ lesion, and 10 HPV+/TERC+ cases showed 6 CIN2+ . In the 14 ASC-H cases, 9 CIN2+ and 2 CIN1 were detected. 9 CIN2+ cases were all TERC+. In the 23 HSIL cases that were histologically confirmed, 2 inflammations, 1 CIN1 and 20 CIN2+ were detected. All of the CIN and SCC cases were TERC+. In the 3 cytological interpreted SCC cases, the HPV and TERC tests showed double-positive results; the histological results were 1 CIN3 and 2 SCC. In cytological diagnoses of NILM, ASCUS, LSIL, ASC-H, HSIL and SCC, the HPV positive rates were 24.1%, 50.0%, 71.4%, 71.4%, 91.7% and 100.0%, respectively; the TERC positive rates were 2.7%, 19.2%, 52.4%, 64.3%, 91.7% and 100.0%, respectively. The TERC positive rates were consistent with the cytological diagnoses. Moreover, significant differences were detected between LSIL or lower and HSIL or higher ($p < 0.01$). The numbers of cases with HPV infections and TERC amplifications of various histological diagnoses are shown in Table 2. Of the 164 histologically confirmed normal cases, the HPV infection rate was 51.2 % (84/164), whereas the TERC positive rate was only 9.2 % (15/164). Of the 29 CIN1 cases, 82.8 % (24/ 29) of the cases had HPV infections, and 17.2 % (5 / 29) of the cases were TERC+. In the 50 CIN2+ cases, the HPV infection rate was 100.0%, whereas the TERC positive rates in CIN2, CIN3 and SCC cases were 76.2%, 100.0% and 100.0%, respectively. An increase of the TERC positive rate was observed as the severity of histology diagnosis increased, and significant difference was observed between the normal/CIN1 cases and the CIN2+ cases (10.4% vs. 90.0%, $p < 0.01$).

Comparison of cytology analysis, HC2 HPV DNA test and TERC amplification test for CIN2+ detection

We calculated the sensitivity, specificity, PPV, NPV, accuracy and referral rate of cytology analysis, HC2 HPV DNA test and TERC amplification test for the CIN2+ diagnosis (Table 3). Youden's index was also calculated to compare the overall accuracy of each method. ASCUS+

Table 3. Comparison of Cytology, HPV DNA Test and TERC Amplification Test According to Various Screening Approaches

Screening Approach	Positive Threshold†	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %	Youden's Index, %	Referral Rate, %
Cytology	ASCUS+	84.0	64.3	37.8	93.9	68.3	48.3	45.7
HPV	HPV+	100.0	44.0	31.7	100.0	55.6	44.0	65.0
TERC	TERC+	90.0	89.6	69.2	97.2	89.7	79.6	26.8
Cytology and HPV Co-testing	ASCUS+ and HPV+	84.0	83.4	56.8	95.3	83.5	67.4	30.5
Cytology and TERC Co-testing	ASCUS+ and TERC+	84.0	93.8	77.8	95.8	91.8	77.8	22.2
HPV and TERC Co-testing	HPV+ and TERC+	90.0	92.2	75.0	97.3	91.8	82.2	24.7
Cytology, HPV and TERC Co-testing	ASCUS+ and TERC+ and HPV+	84.0	94.8	80.8	95.8	92.6	78.8	21.4

†Positive threshold; ASCUS+, determined as ASCUS or higher; HPV+, determined as RLU/CO ≥ 1.0 of a HC2 HPV DNA test; TERC+, determined as ≥ 5% aberrant TERC cells of the cells observed

was taken as cut-off of cytology analysis, RLU/CO ≥ 1.0 was regarded as cut-off value of the HC2 HPV DNA test, and cut-off value of the TERC amplification test was set at ≥ 5% aberrant cells of the cells observed. Among cytology analysis, the HC2 HPV DNA test and TERC amplification test, HC2 HPV DNA test still had the highest sensitivity (100.0%), which was higher than that of the TERC amplification test (90.0%) and the cytology analysis (84.0%) ($p < 0.05$) . However, in terms of specificity, the TERC amplification test was the best (89.6%), higher than the cytology analysis (64.3%) and the HC2 HPV DNA test (44.0%) ($p < 0.05$). The PPV of the TERC amplification test (69.2%) was higher than that of the cytology analysis (37.8%) and the HC2 HPV DNA test (31.7%) ($p < 0.05$). The NPV of the TERC amplification test (97.2%) was higher than that of the cytological analysis (93.9%) but lower than that of the HC2 HPV DNA test (100.0%, $p < 0.05$). The accuracy of the TERC amplification test (89.7%) was higher than that of the cytology analysis (68.3%) and that of the HC2 HPV DNA test (55.6%, $p < 0.05$). Regarding the combined sensitivity and specificity according to the Youden's index, the TERC amplification test had the highest value (79.6%), followed by the cytology analysis (48.3%) and the HC2 HPV DNA test (44.0%). On a contrary trend, the referral rate of the TERC amplification test (26.8%) was lower than that of the cytology analysis (45.7%) and the HC2 HPV DNA test (65.0%) ($p < 0.05$).

Diagnostic performance of various combinations of cytology analysis, HC2 HPV DNA test and TERC amplification test

Diagnostic characteristics of cytology analysis, HC2 HPV DNA test, TERC amplification test and their combinations are listed in Table 3. Among all of the screening approaches, a single HC2 HPV DNA test had the highest sensitivity (100.0%). TERC amplification test used in conjunction with the HC2 HPV DNA test showed lower sensitivity but higher specificity than a single HC2 HPV DNA test (90.0% vs. 100.0%, 92.2% vs. 44.0%, respectively), and highest level of combined sensitivity and specificity among all of the screening approaches (Youden's index 82.2%). Regarding the screening approaches using cytology analysis, co-testing of cytology and TERC had similar sensitivity and higher specificity than co-testing of cytology and HPV (84.0% vs. 84.0%, 93.8% vs. 83.4%, respectively). In comparison with co-testing of cytology and HPV, co-testing of TERC and HPV showed higher sensitivity and specificity (90.0% vs. 84.0%, 92.2% vs. 83.4%, respectively).

Discussion

Liquid-based cytology is the most often used device for cervical cancer screening, whereas limitations exist because the method is subjective and relatively insensitive. The high-risk HPV DNA test is highly sensitive but low specific (Rodríguez et al., 2008; Rosa et al., 2008), and is not effective for distinguishing HPV physical status (episomal or integrated infection). Therefore, it is incapable of identifying which part of patients with the ASCUS or HPV-positive results are likely to have a CIN2+ lesion (Arbyn et al., 2004, Poomtavorn et al., 2011). According to the World Health Organization (WHO) guideline (2006) and the consensus guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP) (Wright et al., 2007), the recommendation for CIN1 cases is to undergo follow-up examinations at defined intervals, whereas the recommendation for CIN2/3 is to undergo immediate treatment. Giving the limitations of the current screening methods, more accurate and reliable biomarkers supply another approach to assist the differential diagnosis (Ekalaksananan et al., 2011; Cheah et al., 2012). The change of the biomarker should be an early event of cervical carcinogenesis. Oncogene amplification is frequently observed in cervical precancerous lesion and is a fairly early event in cervical carcinogenesis.

The pattern of oncogene amplified in cervical cancer is conserved. TERC (3q26) is the RNA component of the human chromosome telomerase, which has been certified to be the most frequently observed amplified oncogene in cervical precancerous lesions (Heselmeyer et al., 1996, 1997; Kirchhoff et al., 1999; Umayahara et al., 2005). Heselmeyer et al. (2003) applied a FISH probe set to the cervical cytological specimens and found that the TERC amplification status was associated with the severity of cervical lesions, and was predictive for the disease regression or progression (Heselmeyer et al., 2005). In our study, the TERC positive rates were 9.2 % in normal cases, 17.2% in CIN1 cases, 76.2% in CIN2 cases and 100.0% in CIN3 and SCC cases, with a significant difference between CIN1/lower and CIN2/higher ($p < 0.05$). Because most of the cases with negative results and a minority of the cases with positive results (most of them are HPV-positive only) were not histologically

confirmed, lost of some normal histological results gives an explanation that no significant difference was observed between the normal group and CIN1 group. A similar trend was observed in cytological diagnoses with a significant difference of TERC positive rates between LSIL/lower and HSIL/higher ($p < 0.05$). These results demonstrate that the TERC positive rates increase with the cytological and histological grades, especially between normal/low-grade and high-grade lesions. These findings support the results reported by other investigators (Heselmeyer et al., 1996, 1997, 2003, 2005; Kirchhoff et al., 1999; Umayahara et al., 2005; Sokolova et al., 2007; Policht et al., 2010).

The high-risk HPV DNA is widely recognized to integrate into the human genome and lead to genomic instability, such as amplification of the TERC gene, which is crucial in the malignant transformation of cervical cells. Hopman et al. (2006) found that the oncogenic HPV integration and the TERC expression are two associated genetic events in carcinogenesis. In the present study, TERC positive rate was higher in the HPV-positive cases than in the HPV-negative cases (37.5% vs. 2.0%, $p < 0.01$). The correlation index between the TERC amplification and the HPV infection was 0.50 ($p < 0.01$). This finding supports the opinion that the TERC amplification is corresponded with the HPV infection. Because not all of the HPV infections are integrated ones and will lead to TERC amplifications, the TERC amplification rate is lower than the HPV infection rate. That's why the TERC amplification test is more specific than the HC2 HPV DNA test. In the present study, among cytology analysis, HC2 HPV DNA test and TERC amplification test, the HC2 HPV DNA test showed the highest sensitivity (100.0%) and NPV (100.0%), but the lowest specificity (44.0%) and PPV (31.7%). The TERC amplification test had the highest specificity (89.6%) and PPV (69.2%) of the three methods, and compensated for the limitations of the HC2 HPV DNA test.

The application of the HPV DNA test for cervical cancer screening remains controversial. Ronco et al. (2010) found that the HPV-based screening is more effective than cytology in preventing invasive cervical cancer. Katki HA et al. (2011) considered that a single negative HPV test was sufficient to reassure against cervical cancer over 5 years. Testing for HPV without adjunctive cytology might be sufficiently sensitive for primary screening for cervical cancer. Management of women with high-risk HPV infections and normal cytological results is also a hot spot. 7.9% women will have positive cytological results within 4 years without consideration of past screening results (Castle et al., 2011). Update of the ASCCP guideline recommended these cytological negative/HPV positive cases to be triaged by high-risk HPV typing (Apgar et al., 2009). The guideline was validated by a study of the ATHENA (Addressing THE Need for Advanced HPV Diagnostics) Study Group (Wright et al., 2011). In the present study, we compared the diagnostic characteristics of cytology analysis, HC2 HPV DNA test, TERC amplification test and their combinations to explore an optimal strategy for cervical cancer screening. TERC amplification tested in conjunction with the HC2 HPV DNA test showed a combination of 90.0% sensitivity and 92.2% specificity, which was the highest among all of the screening approaches. The TERC amplification test increased the specificity and maintained the high sensitivity of the HC2 HPV DNA test. Compared with cytology and HPV co-testing that was commonly used, the HPV and TERC co-testing showed higher sensitivity and specificity (90.0% vs. 84.0%, 92.2% vs. 83.4%, respectively).

It would be of great interest to explore the reason of discordance between the FISH test results and the cytological or histological results. Of the 50 histologically confirmed CIN2+ lesions in the present study, 5 cytology negative (NILM) and TERC negative cases were noticed. We reviewed the colposcopic and histological images and found that most of them were focal CIN2+ cases, and therefore the sampling omissions were not excluded. From the 3 cases that were NILM but TERC positive, higher sensitivity of the TERC amplification test than the cytological analysis was observed. This could be attributed to the fact that the abnormal cell counts were small and overlooked, or the morphological changes had not been manifested.

Carcinogenesis of cervical cancer is a process that begins with the integrated HPV infection, and is followed by the genotype and phenotype change. The morphological screening that relies on cytology analysis is actually a phenotype diagnosis, and usually causes misdiagnosis of cases that are genotypically positive but phenotypically negative. Using as a complementary procedure, the HC2 HPV DNA test is unable to discriminate which part of patients are at high risk of developing CIN2+ lesions. The TERC amplification test can detect normal-look cells with amplified oncogenes, and provide important indications for diagnosis. However, the TERC amplification test had lower sensitivity than the HC2 HPV DNA test, and therefore a small fraction of CIN2+ cases can be undiagnosed (Voss et al., 2010). Combination of the TERC amplification test and the HC2 HPV DNA test compensate for the shortcomings of the two tests, and provide a clinically applicable diagnostic approach with higher combined sensitivity and specificity for cervical cancer screening.

Acknowledgements

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