

Detection of *TERC* Amplification in Cervical Epithelial Cells for the Diagnosis of High-Grade Cervical Lesions and Invasive Cancer

A Multicenter Study in China

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Because the activation of telomerase is a relatively early event in the progression of cervical carcinogenesis, the expression of the human telomerase RNA gene, *TERC*, has the potential to serve as a biomarker for both the diagnosis and prognosis of cervical neoplasias. In total, 83 research centers participated in the study, and 7786 patients were enrolled. *TERC* amplification was detected using a dual-color fluorescence *in situ* hybridization (FISH) probe set, and these results were compared with cytological and histological results, testing for high-risk human papillomavirus (HPV) DNA ($n = 2316$ for the HPV DNA test), as well as patient age. *TERC* amplification was found to be increased in more advanced cases of cervical carcinogenesis. Moreover, a Youden's index value and the area under the receiver operating characteristic (ROC) curve were also calculated for samples with *TERC* amplification and found to be higher than the same values calculated for both cytology and high-risk HPV analyses of the same samples. With regard to cytological ASCUS and LSIL findings, the combination of HPV + *TERC* testing showed the potential to provide effective triaging to detect CIN2⁺. Therefore, *TERC* amplification represents a valuable genetic biomarker, which in combination with an evaluation of cytology or HPV testing, can achieve higher sensitivity and specificity in distinguishing high-grade cervi-

cal lesions and invasive cancers from low-grade lesions compared with conventional methods. (*J Mol Diagn* 2010, 12:808–817; DOI: 10.2353/jmoldx.2010.100021)

Cervical cancer is the second most common cancer in women, and in 2005, more than 250,000 deaths worldwide were the result of cervical cancer.¹ Because ~80% of all cervical cancer cases are diagnosed in developing countries, in China, there are ~135,000 new cases and 50,000 deaths each year due to this disease.² Without improvements in the treatments available, the number of cases of cervical cancer are projected to rise by almost 25% over the next 10 years. Therefore, to decrease the incidence and mortality associated with cervical cancer, effective approaches are needed to facilitate the screening and diagnosis of cervical precancerous lesions, especially high-grade lesions.

The use of cervical cytopathologic examinations has reduced the incidence of cervical cancer in many countries,^{3,4} however the low sensitivity and high variability of this method has limited its significance.⁵ With human papillomavirus (HPV) being the main cause of cervical cancer, detection of HPV DNA has become an important supplement to cytologic evaluations performed during cervical cancer screenings.^{6,7} However, most women who are infected with HPV eliminate the virus and do not progress to high-grade disorders, thereby decreasing the specificity of HPV DNA tests for the detection of cervical cancer.⁸

The infection and integration of HPV into epithelial cells are key steps in the induction of malignant alterations in cervical cancer; however, there are additional genomic events that also occur.⁹ One of the events associated with cervical cancer is an increase in copy number of the long arm of chromosome 3 (3q).¹⁰ Gain of 3q in cervical

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cancer has been mapped to 3q26-27, a region which contains the human *TERC* gene that encodes the template of telomerase RNA.¹¹ Because telomerase activation is a relatively early event in cervical carcinogenesis, telomerase activity and expression of its components may represent valuable biomarkers for the diagnosis and prognosis of patients with cervical neoplasia.¹²

Previous studies have shown that detection of *TERC* amplification might be a valuable biomarker for the detection of cervical lesions.¹³⁻¹⁵ We have also investigated this issue since 2007 and have found that detection of *TERC* amplification by fluorescence *in situ* hybridization (FISH) has the potential to be an effective approach for the differential diagnosis of cervical disorders.^{16,17} Despite this work, the association between *TERC* amplification and cervical cytopathology, histopathology, HPV infection status, and patient age remains unclear. Importantly, the sensitivity and specificity of detecting *TERC* amplification remains unclear. Therefore, in this study, *TERC* amplification, in combination with cytopathology and HPV testing, was performed to detect cervical disorders, and these results were compared with histopathological evaluations of the samples. Moreover, the clinical usefulness of detecting *TERC* amplification to identify patients with cervical precancerous lesions was investigated.

Materials and Methods

Study Centers and Patients

In total, 7786 women from 83 research centers in China were recruited for this multicenter study, and informed consent was obtained from each patient before enrollment. The centers involved represent hospitals located in various parts of China, including 29 provinces, autonomous regions, and municipalities. All of the hospitals included were considered tier 1 and had the capacity to perform liquid-based thin-layer cytopathologic evaluations, colposcopy, biopsies, histopathological examinations, and FISH analysis, and were found to be consistent in their analyses. In addition, 1033 women included in this study were also previously analyzed in another study.¹⁶

The women included in this study ranged in age from 18 to 93 years (mean, 39.7 ± 9.7) and visited an outpatient clinic between June 2007 and May 2009. These patients either underwent routine screening or were returning for evaluation based on an abnormal cervical cytology result, HPV results, or to have symptoms of increased leucorrhea discharge or postcoital bleeding examined. All patients were checked to exclude acute pelvic infection and pregnancy, and liquid-based, thin-layer, cytopathologic examinations were performed using ThinPrep (Cytec Corp, Boxborough, MA) or AutoCyte (AutoCyte, Burlington, NC) systems. FISH analysis was also performed using a *TERC*-specific probe in blinded assays. A colposcopy, biopsy, and histopathological examination were conducted as needed. For some patients, high-risk HPV DNA was detected using the Digene Hybrid Capture 2 method (HC2) (Qiagen, Gaithersburg,

MD) depending on the institution at which the patient was treated. This project was approved by the ethical committee of Peking University People's Hospital, which played the leading role in directing this research.

Cervical Cytopathologic Evaluation

Cervical cells were collected from the squamocolumnar junction of the patient's cervix using a sampling brush and the cells were stored in Thin-prep or Autocyte cytologic test reserving fluid. Cells were subsequently prepared on slides and screened by cytotechnologists. Grading of all 7786 cases was performed according to the 2001 revision of the Bethesda system³ and included the following: cases negative for intraepithelial lesion or malignancy (NILM; 1958 cases, 25.1%), cases of benign cellular changes (29 cases, 0.4%), atypical squamous cells of undetermined significance (ASCUS; 1985 cases, 25.5%), atypical squamous cells that cannot exclude a high-grade lesion (ASC-H; 87 cases, 1.1%), low-grade squamous intraepithelial lesions (LSIL; 1824 cases, 23.4%), high-grade squamous intraepithelial lesions (HSIL; 1900 cases, 24.4%), and atypical glandular cells (AGC; 3 cases, 0.04%). For the purpose of this study, calculations of sensitivity, specificity, and positive and negative predictive values were performed with a positive cytology test defined as a result \geq HSIL.

Cervical Histopathological Examination

Colposcopy was performed on 6726 patients who had a cytology result of ASCUS or greater, had a positive high-risk HPV result, or had apparent symptoms. All of the colposcopists and histopathologists received uniform training before the start of this study, with colposcopists following a standardized protocol. Four-quadrant biopsy, or abnormal-appearing biopsy areas, were collected and biopsy specimens were reported using terminology associated with the grading of cervical intraepithelial neoplasia.¹⁸ To avoid discrepancies among different centers as much as possible, questionable slides were delivered to the central laboratory in Beijing for a final decision.

High-Risk HPV DNA Test

There were 25 hospitals in the study that were able to perform high-risk HPV DNA tests using the HC2 method. HPV samples were obtained from 2316 women at the same time that cytology specimens were collected, according to the HC2 protocol. Levels of HPV DNA >1 pg/ml were considered a positive result.

FISH Analysis

After the liquid-based cytological preparations were completed, the remaining cervical cells were used for FISH analysis. Briefly, cells were centrifuged at $1600 \times g$ for 6 minutes, washed twice with $1 \times$ PBS, then incubated with collagenase B at 37°C for 20 minutes. Samples were

Table 1. *TERC* Amplification Rate in Cases with Different Cytological Results ($n = 7786$)

Cytological results	<i>TERC</i> amplification rate, % (cases)
NILM	10.1 (198/1958)
Benign cellular change	31.0 (9/29)
ASCUS	34.5 (684/1985)
LSIL	43.1 (786/1824)
ASC-H	60.9 (53/87)
HSIL	82.0 (1558/1900)
AGC	100 (3/3)

then centrifuged again, incubated with deionized water at 37°C for 30 minutes, centrifuged, and cells were resuspended in 5 ml 0.075 mol/L KCl. After another incubation for 20 minutes at 37°C, cells were fixed at RT for 10 minutes with 5 ml methanol-acetic acid (3:1), centrifuged, then smeared with 1–2 ml methanol-acetic acid onto slides pretreated with 3-aminopropyltriethoxysilane.

The dual-color FISH probe set was produced and provided by GP Medical Technologies, Ltd. (Beijing, China). This probe set had previously been approved by the State Food and Drug Administration of China and accepted for clinical application in China. Chromosome 3q26.3 is the binding site for the *TERC* probe, and details of the probe set and its conditions have previously been described.^{16,17} Before hybridization, slides were subjected to pepsin digestion and dehydrated in an ethanol series. The slides and probe mixture were denatured separately in 70% formamide/2× standard saline citrate (SSC) at 73 ± 1°C for 5 minutes. After hybridization in a humid environment overnight at 37°C, slides were washed with 50% formamide/2× SSC, 2× SSC, and 2× SSC/0.1% NP40, then counterstained with 4,6-diamidino-2-phenylindole (DAPI).

Hybridized slides were evaluated using a fluorescent microscope by uniformly trained personnel. Slides were analyzed independently by two observers at each center, and at least 100 nuclei were evaluated for each specimen. The signal ratio of CSP3 to *TERC* at 2:2 in a cell indicated a normal signal pattern, whereas ratios of 2:3, 2:4, 2:5, 3:3, 4:4, and so on, represented abnormal signal patterns. Therefore, an abnormal signal pattern was recorded if more than two *TERC* signals were observed. For each medical center, the mean and three times SD of the percentage of nuclei with a combination of all of the abnormal signal patterns possible were calculated as the cut-off value. Cervical epithelial cells from 20 individ-

Table 2. *TERC* Amplification Rate in Cases with Different Histopathological Results ($n = 6726$)

Histopathological results	<i>TERC</i> amplification rate, % (cases)
Normal	6.2 (11/178)
Inflammatory	10.5 (113/1079)
CIN1	20.8 (428/2054)
CIN2	68.6 (952/1387)
CIN3	82.4 (1162/1410)
SCC	94.9 (556/586)
Glandular carcinoma	87.5 (28/32)

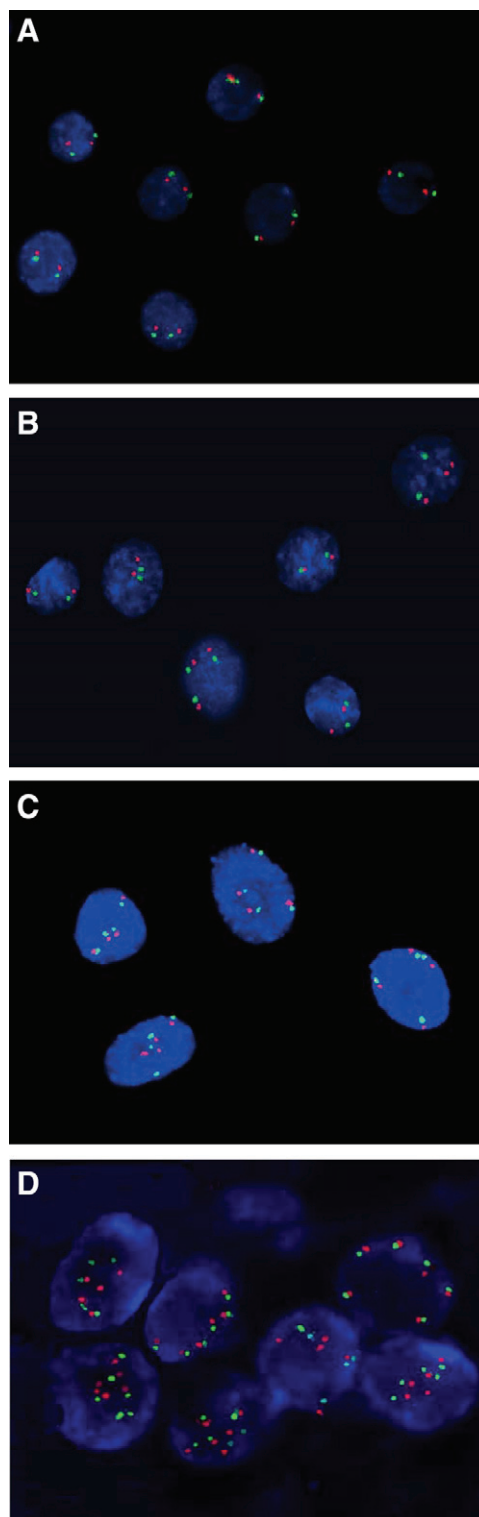


Figure 1. Representative immunofluorescence images of different cell types stained for *TERC* and CSP3. **A:** Normal cells with a CSP3 (green):*TERC* (red) signal ratio of 2:2. **B:** A few CIN1 cells with 2:3 or 3:3 signal patterns. **C:** CIN3 cells exhibiting a 4:4 signal pattern. **D:** SCC cells with 5:5 and 6:6 signal patterns. Magnification, ×1000.

uals with normal cytopathologic examinations and negative HPV tests were used to establish the cut-off value. For samples positive for *TERC* amplification, the percentage of nuclei having a combination of abnormal signal

Table 3. Sensitivity, Specificity, Positive Prediction Value (PPV), Negative Prediction Value (NPV), and Area under ROC Curve of Cytology (\geq HSIL), High-Risk HPV, and *TERC* Amplification Analyses for the Diagnosis of High-Grade Cervical Lesions and Invasive Cancer ($n = 2316$)

Characteristic	Cytology	High-risk HPV	<i>TERC</i>
Sensitivity, % (95%CI)	53.9 (51.0–56.8)	91.3 (89.7–92.9)	80.7 (78.4–83.0)
Specificity, % (95%CI)	93.3 (91.9–94.7)	39.3 (36.5–42.1)	83.8 (81.7–85.9)
PPV, % (95%CI)	88.6 (86.2–91.0)	59.2 (56.9–61.5)	82.8 (80.6–85.0)
NPV, % (95%CI)	67.7 (65.5–70.0)	82.4 (79.2–85.5)	81.9 (79.7–84.0)
Youden's index, % (95%CI)	47.2 (44.0–50.4)	30.6 (27.3–33.8)	64.5 (61.4–67.6)
Area under ROC curve, % (95%CI)	73.6 (71.5–75.7)	65.3 (63.0–67.5)	82.3 (80.5–84.1)
Kappa value, % (95%CI)	47.5 (44.2–50.8)	30.3 (27.0–33.6)	64.6 (61.5–67.7)

patterns should be more than the cut-off value. In this study, the mean cut-off value determined for all of the participating centers was $6.4 \pm 2.3\%$. Correspondingly, 6.4% was established as the standard cut-off value for interpreting the testing results from all study centers.

Statistical Analysis

All of the data collected were entered in a database by a data-entry agency (Likang, Beijing, China) and analyzed uniformly. The relationship between *TERC* amplification, HPV analyses, as well as cytology and histology diagnoses, were evaluated using the χ^2 test. Spearman and Kendall's coefficients, as well as Kappa values, were also calculated. To account for a combination of increased sensitivity and decreased specificity, a Youden's index value ($Y = \text{sensitivity} + \text{specificity} - 1.0$) was calculated to compare the overall accuracy of each method.^{19,20}

All analyses were performed using SPSS 13.0 (SPSS, Chicago, IL), and receiver operating characteristic (ROC) analysis was conducted using STATA 9.0 (StataCorp LP, College Station, TX). *P* values less than 0.05 were considered statistically significant.

Results

Of the 7786 cases of cervical samples evaluated, *TERC* amplification rate in cases with different cytological results was shown in Table 1. For the ASCUS, LSIL, ASC-H, and HSIL cases, a pairwise significant difference was detected ($P < 0.01$ in each case). Of the 6726 cases where colposcopy and histopathological evaluations were performed, *TERC* amplification rate in cases with different histopathological results was shown in Table 2. Among the cases labeled as normal, with inflammation, as cervical intraepithelial neoplasia (CIN)1–3, and as squamous cervical cancer (SCC), significant differences in each pairwise comparison were detected ($P < 0.01$ in each case). Interestingly, the number of abnormal cells present and the complexity of the abnormal signal patterns observed increased with the severity of the cervical disorders evaluated (Figure 1, A–D).

In 2316 cases tested for high-risk HPV DNA, 75.7% (1754/2316) were positive, including 27.3% (12/44) of histologically normal specimens, 42.2% (144/341) of inflammatory specimens, 70.5% (560/794) of CIN1 cases, 88.1% (406/461) of CIN2 cases, 93.9% (490/522) of CIN3 cases, 92.7% (140/151) of SCC cases, and 66.7% (2/3) of

glandular carcinoma cases. Overall, the positive rate for *TERC* amplification was 57.2% (1003/1754) for HPV-positive cases, and only 18.9% (106/562) for HPV-negative cases, and this difference was statistically significant ($P < 0.001$). Moreover, a positive correlation between *TERC* amplification and detection of HPV was observed and associated with a Spearman correlation coefficient of 0.329 ($P < 0.001$).

To better understand the capacity for different methods to evaluate high-grade cervical lesions (ie, CIN2/3) and invasive cancers, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cytology, high-risk HPV detection, and *TERC* amplification analyses were calculated (Table 3). High-risk HPV DNA testing was associated with the highest levels of sensitivity (91.3%), followed by *TERC* amplification (80.7%) and cytology analyses (53.9%). However, in terms of specificity, cytology evaluations were the best (93.3%), followed by *TERC* amplification (83.8%) and high-risk HPV DNA analyses (39.3%), with significant differences detected between each ($P < 0.05$). The PPVs of *TERC* amplification (82.8%) and cytology analyses (88.6%) were not statistically different, although both were significantly higher than that of high-risk HPV testing (59.2%). Moreover, the NPV of *TERC* amplification analysis (81.9%) was similar to that of high-risk HPV testing (82.4%), yet higher than that for cytological evaluations (67.7%). Regarding the analysis according to Youden's index and the area under the ROC curve, detection of *TERC* amplification had the highest values (see also Figure 2). A kappa value was also calculated to determine the uniformity of the three methods in evaluating the histological classification of each sample, and detection of *TERC* amplification was found to have the highest value.

The correlation between *TERC* amplification and patient age was determined. Four age groups were established that included patients of 18–29 years (353 cases), 30–49 years (1679 cases), 50–64 years (263 cases), and 65+ years (21 cases). The positive rate of *TERC* amplification was found to increase with patient age and was associated with a Kendall's correlation coefficient of 0.070 ($P < 0.001$). The ability of different methods to detect high-grade cervical lesions in different age groups is shown in Table 4 and Figure 3 (A–C). *TERC* amplification provided the highest sensitivity for patients 30–64 years old (81.6–82.0%), whereas the best specificity and NPV were associated with patients 18–29 years old (88.7% and 85.3%, respectively). The highest PPV was detected for patients older than 65

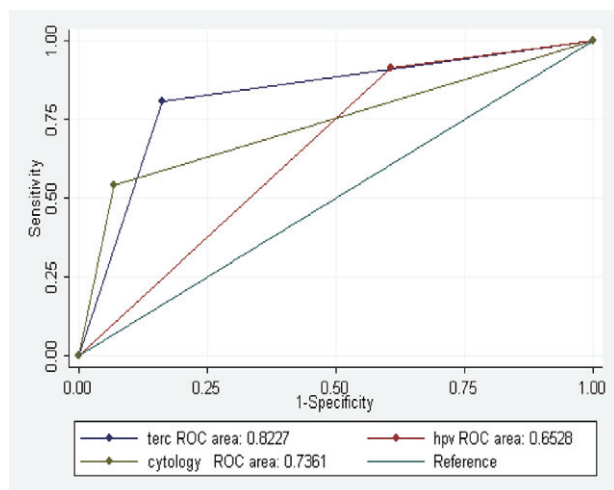


Figure 2. ROC curves for detection of CIN2⁺ by cytology, high-risk HPV DNA testing, and *TERC* amplification analysis.

years old (91.7%). Within the liquid-based cytology examinations, the highest specificity was associated with patients 18–29 years old (95.5%), and the highest PPV was detected for patients 30–49 years old (89.6%). For samples tested for high-risk DNA, very good levels of sensitivity were associated with the 18- to 64-year-old group (91.0–91.6%), and the highest NPV was detected for the 18- to 29-year-old group (85.7%). However, there was no statistically significant difference between each pairwise comparison.

Finally, the capacity for *TERC* amplification, in combination with detection of high-risk HPV DNA, to identify histological CIN2⁺ or CIN3⁺ patients among cytological ASCUS and LSIL cases was analyzed. Of the 660 patients with cytological ASCUS and 601 patients with LSIL, all underwent an HPV test, colposcopy-directed biopsy, and *TERC* amplification examination. Of these, 196 (29.7%) samples were verified to be histological CIN2⁺, 97 (14.7%) samples were CIN3⁺ in cytological ASCUS, and 233 (38.8%) samples were verified to be histological CIN2⁺, 105 (17.5%) samples were CIN3⁺ in cytological LSIL women. The sensitivity of detecting CIN2⁺ or CIN3⁺ by *TERC* amplification was lower than that for HPV testing in both ASCUS and LSIL cases, yet the specificity associated with the detection of *TERC* amplification was higher than that for HPV testing (at least $P < 0.05$). The NPV of *TERC* testing was also found to be similar to that of HPV testing ($P > 0.05$), whereas if *TERC* and HPV

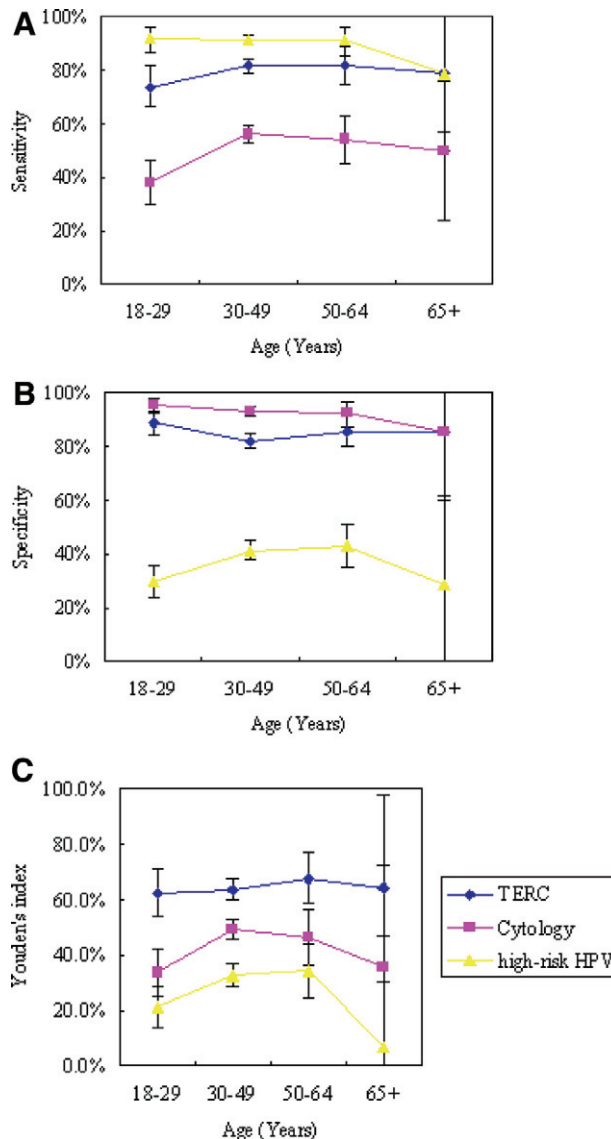


Figure 3. Graphs of the sensitivity (A), specificity (B), and Youden's index values (C) calculated for the methods of cytology, high-risk HPV DNA detection, and *TERC* amplification analyses for the detection of high-grade cervical lesions and invasive cancer in different age groups. Bars represent 95% confidence intervals.

testing were performed on the same sample, the specificity and NPV of these methods for detecting CIN2⁺ was significantly higher than for either test alone (at least $P < 0.05$). Moreover, no significant decrease in specificity

Table 4. Cytology (≥HSIL), High-Risk HPV, and *TERC* Amplification Analyses in Association with Different Age Groups for the Detection of High-Grade Cervical Lesions and Invasive Cancer ($n = 2316$)

Characteristic	Cytology				High-risk HPV	
	18–29	30–49	50–64	65+	18–29	30–49
Sensitivity, % (95%CI)	38.2 (66.5–81.6)	56.3 (53.0–59.6)	54.1 (45.3–62.9)	50.0 (23.8–76.2)	91.6 (86.9–96.4)	91.5 (89.6–93.3)
Specificity, % (95%CI)	95.5 (92.8–98.2)	93.0 (91.2–94.7)	92.2 (87.8–96.6)	85.7 (59.8–100.0)	29.7 (23.7–35.7)	41.3 (37.9–44.7)
PPV, % (95%CI)	83.3 (73.9–92.8)	89.6 (87.0–92.1)	85.7 (77.9–93.5)	87.5 (64.6–100.0)	43.5 (37.6–49.3)	62.6 (60.0–65.3)
NPV, % (95%CI)	72.4 (67.2–77.5)	66.4 (63.7–69.2)	69.9 (63.3–76.5)	46.2 (19.1–73.3)	85.7 (77.9–93.5)	81.9 (78.1–85.6)
Youden's index, % (95%CI)	33.7 (24.9–42.4)	49.3 (45.5–53.0)	46.3 (36.4–56.2)	35.7 (0–72.6)	21.3 (13.7–29.0)	32.8 (28.9–36.6)

(table continues)

Table 5. Sensitivity, Specificity, and NPV of High-Risk HPV, *TERC* Amplification, and High-Risk HPV + *TERC* Amplification Analyses for the Diagnosis of CIN2⁺ in Cytologic ASCUS and LSIL Women

Characteristic	Cytology (n)	High-risk HPV	<i>TERC</i>	High-Risk HPV + <i>TERC</i>
Sensitivity, % (95%CI)	ASCUS (660)	89.3 (85.0–93.6)	75.5 [†] (69.5–81.5)	96.4 [†] (93.8–99.0)
	LSIL (601)	95.3 (92.6–98.0)	75.5 [†] (70.0–81.1)	99.1* (98.0–100.0)
Specificity, % (95%CI)	ASCUS (660)	45.5 (40.9–50.0)	86.9 [†] (83.8–89.9)	41.6 (37.1–46.1)
	LSIL (601)	23.4 (19.1–27.7)	78.5 [†] (74.3–82.7)	21.2 (17.0–25.4)
NPV, % (95%CI)	ASCUS (660)	91.0 (87.3–94.6)	89.4 (86.5–92.2)	96.5* (94.0–99.1)
	LSIL (601)	88.7 (82.4–95.0)	83.5 (79.6–87.4)	97.5* (94.1–100.0)

Statistically different from high-risk HPV test **P* < 0.05, [†]*P* < 0.01.

was observed (Table 5). Similarly, the specificity associated with detecting CIN3⁺ by assaying both *TERC* amplification and HPV DNA was significantly higher than performing either test alone (at least *P* < 0.05). Moreover, the NPV for the combined testing was higher than that for detection of *TERC* amplification (*P* < 0.05) yet was similar to the NPV of HPV testing alone (*P* > 0.05, Table 6).

Discussion

Most patients with CIN1 regress spontaneously, whereas a few patients persist or advance to high-grade lesions. Therefore, from a clinical management point of view, it is crucial to accurately identify patients with high-grade lesions (CIN2/3) to appropriately treat them as soon as possible to prevent the progression to carcinoma. Despite the success of cytologic screenings provided as a public health service to reduce the incidence and mortality of cervical cancer, a single cytologic testing is relatively insensitive, poorly reproducible, and frequently yields equivocal results.²¹ Because more than 95% of cervical cancer biopsies contain oncogenic HPV,²² an HPV test represents a very sensitive method for discerning lesions that have low versus high risk for progression. However, the low specificity associated with HPV testing has limited its clinical application.⁸ Therefore, the search for more effective biomarkers and treatment strategies for managing cervical disorders remains.

In 2003, Heselmeyer-Haddad et al²¹ first studied the genomic amplification of *TERC* in 57 thin-layer cervical cytologic slides and found that 63% of HSIL (CIN2) lesions and 76% of HSIL (CIN3) lesions contained extra copies of chromosome 3q. Several authors subsequently proposed that amplification of chromosome 3q was associated with higher grade cervical disorders, with

0–15.4% of 3q amplifications detected in normal cytology slides, 13% in ASCUS slides, 0 to 70% in LSIL slides, 25% in ASC-H slides, and 62.5 to 100% in HSIL slides.^{13–15,21} Correspondingly, these data suggested that *TERC* amplification could be used to screen for cervical lesions. Although the results of these reports were relatively consistent, most of the values varied greatly, probably due to the small sample size (<70 cases) analyzed in each of these studies.

We previously reported that the positive rate of *TERC* amplification in an analysis of 1033 cases increased with progression of severity of cervical lesions.¹⁶ These cases are included in this study where we present the largest sample size to date analyzing the role of *TERC* amplification. *TERC* amplification was detected in 10.1% of patients with cytological NILM, in 31.0% of patients with benign cellular changes, in 34.5% of patients with ASCUS, in 43.1% of patients with LSIL, in 60.9% of patients with ASC-H, and in 82.0% of cases with HSIL. These results further confirm that the positive rate of *TERC* amplification increases with more advanced cervical disorders.

Because histopathological evaluation is considered the gold standard for determining the presence of cervical lesions,²³ *TERC* amplification was also compared with histological analysis in this study. Detection of *TERC* amplification produced a similar pattern of identification as cytological evaluations, with *TERC* amplification detected in 6.2% of normal cases, 10.5% of inflammatory cases, 20.8% of CIN1 cases, 68.6% of CIN2 cases, 82.4% of CIN3 cases, and 94.5% of invasive cancers. These results clearly demonstrate that the proportion of cases with *TERC* amplification increased with the grade of lesion analyzed, especially between CIN1 and CIN2 cases (20.8% versus 68.6%, respectively). Furthermore, these results strongly support the use of *TERC* amplification in

Table 4. *Continued*

50–64	65+	<i>TERC</i>			
		18–29	30–49	50–64	65+
91.0 (85.9–96.1)	78.6 (57.1–100.0)	74.0 (66.5–81.6)	81.6 (79.0–84.2)	82.0 (75.1–88.8)	78.6 (57.1–100.0)
43.3 (35.1–51.4)	28.6 (0–62.0)	88.7 (84.6–92.9)	82.1 (79.4–84.7)	85.8 (80.1–91.6)	85.7 (59.8–100.0)
58.1 (51.1–65.1)	68.8 (46.0–91.5)	79.5 (72.3–86.7)	83.0 (80.5–85.6)	83.3 (76.7–90.0)	91.7 (76.0–100.0)
84.7 (76.4–93.0)	40.0 (0–82.9)	85.3 (80.7–89.9)	80.6 (77.9–83.3)	84.6 (78.7–90.5)	66.7 (35.9–97.5)
34.2 (24.6–43.9)	7.1 (0–46.9)	62.8 (54.2–71.4)	63.7 (60.0–67.4)	67.8 (58.9–76.7)	64.3 (30.6–98.0)

Table 6. Sensitivity, Specificity, and NPV of High-Risk HPV, *TERC* Amplification, and High-Risk HPV + *TERC* Amplification Analyses for the Diagnosis of CIN3⁺ in Cytologic ASCUS and LSIL Women

Characteristic	Cytology (n)	High-risk HPV	<i>TERC</i>	High-risk HPV + <i>TERC</i>
Sensitivity, % (95%CI)	ASCUS (660)	92.8 (87.6–97.9)	82.5* (74.9–90.0)	99.0* (97.0–100.0)
	LSIL (601)	96.2 (92.5–99.9)	81.0† (73.4–88.5)	100.0* (100.0–100.0)
Specificity, % (95%CI)	ASCUS (660)	40.0 (35.9–44.0)	77.1† (73.6–80.6)	35.4 (31.4–39.3)
	LSIL (601)	18.8 (15.3–22.2)	65.7† (61.6–69.9)	16.1 (12.9–19.4)
NPV, % (95%CI)	ASCUS (660)	97.0 (94.8–99.2)	96.2 (94.5–98.0)	99.5† (98.5–100.0)
	LSIL (601)	95.9 (91.9–99.8)	94.2 (91.8–96.7)	100.0† (100.0–100.0)

Statistically different from high-risk HPV test **P* < 0.05, †*P* < 0.01.
 Statistically higher than *TERC* amplification ‡*P* < 0.05.

combination with cytological and histopathological evaluations for the differential diagnosis of low-grade versus high-grade cervical disorders.

The transition of an episomal virus to an integrated oncogenic virus is a risk associated with the progression of cervical dysplasia to invasive cancer and can lead to genomic instability including *TERC* amplification.¹¹ In the present study, the rate of *TERC* amplification was found to be much higher in HPV-positive cases than in HPV-negative cases, and the presence of *TERC* amplification corresponded with the presence of HPV infection. Because *TERC* amplification represents an aspect of genomic instability that may develop in the early stages of cancer, it would be predicted to be more specific than the detection of HPV in evaluating morphological changes as a result of cervical disorders.¹⁶ In the present study, both the specificity and PPV of *TERC* amplification for the diagnosis of high-grade cervical lesions were higher than the values associated with the detection of high-risk HPV DNA. Given the similar NPV value and higher PPV value associated with *TERC* amplification analysis versus high-risk HPV DNA detection, the former may have an advantage in monitoring the recovery or recurrence of cervical lesions posttherapy.

The Youden's index value and area under the ROC curve calculated for samples with *TERC* amplification were higher than the same values calculated for cytology and high-risk HPV analyses. These results imply that detection of *TERC* gene amplification by FISH could improve the sensitivity and specificity of diagnosing high-grade cervical lesions and invasive cancer, and this would be consistent with other recent reports.²⁴ Furthermore, detection of *TERC* amplification has been shown to be more consistent with histopathological diagnoses at the highest Kappa value. In the literature, individual studies have evaluated cytology using different thresholds. We also calculated the accuracy of the cytology method by using ASCUS as the cut-off point. As a result, the specificity decreased dramatically to 21.6% despite the level of sensitivity increasing to 93.1%. Overall, the combined sensitivity and specificity levels associated with the cytology analysis using HSIL as a threshold was higher than the sensitivity and specificity associated with ASCUS as a cut-off value. Based on these data, we selected HSIL as the cut-off point for this study, and the accuracy of liquid-based cytology was found to be similar to that reported in Europe and North America, with a sensitivity for detecting high-grade lesions ranging from 42.2–56.4%, and a specificity ranging from 96.3–99.1%.^{5,25} Therefore, from a

clinical standpoint, it appears that detection of *TERC* amplification, in conjunction with cytology and/or high-risk HPV DNA testing, can improve the detection of high-grade cervical lesions and invasive cancer.

When the positive rate of *TERC* amplification was compared with patient age, increased levels of amplification were associated with older patients, and this is consistent with the observation that the severity and invasiveness of cervical diseases increases with age. *TERC* amplification had the highest sensitivity for patients between the ages of 30–64 years, thereby suggesting that detection of *TERC* amplification could improve the detection of moderate and severe cervical precancerous lesions in women of child-bearing age, or closely after the onset of menopause. *TERC* amplification had the highest specificity for the 18- to 29-year-old women, which could be helpful for avoiding overtreatment on young women in this age group. In contrast, Cuzik et al⁵ found that the sensitivity of HPV testing was uniformly high for all ages of women during cervical screening, and the sensitivity of cytology examination was better for women older than 50 years old, with the specificity for both methods increasing with patient age. In this study, the sensitivity of HPV testing and the specificity of cytology examination were both high for patients between 18 and 64 years old, however, both values dropped greatly when the women evaluated were greater than 65 years old. Interestingly, however, detection of *TERC* amplification maintained good sensitivity and specificity for patients 65 years and older. Similarly, the Youden's index value for detection of *TERC* amplification was consistently high for all age groups, while the highest Youden's index value for cytology was associated with the 30–49 age group, and with HPV testing in the 50–64 age group. To our knowledge, this study may represent the first study to evaluate the association between *TERC* amplification and patient age.

Recently, determination of *TERC* amplification by FISH was found to serve as a persistence-progression indicator in LSIL cases,²⁶ and the NPV of *TERC* amplification during the development of CIN2/3 reached as high as 93–100% after an average follow-up period of 12–41 months.²⁷ In this study, the combination of detecting *TERC* amplification and testing for high-risk HPV DNA helped to triage ASCUS and LSIL cytologic findings. For example, the sensitivity of detecting CIN2⁺ using HPV + *TERC* testing was 96.4% in the ASCUS cytologic group and 99.1% in the LSIL group, with NPV values of 96.5% and 97.5%, respectively. Furthermore, all of the above

figures were significantly higher for the combined testing approach than for detection using either approach alone. For an ASCUS cytologic diagnosis, HC2 testing for high-risk HPV types has previously been shown to be a sensitive triage method for the detection of CIN2⁺.²⁸ In our results, detection of *TERC* amplification further increased the sensitivity and NPV of HC2 without decreasing its specificity to detect CIN2⁺ in high-risk women. With regard to cytologic LSIL findings, neither a single HPV test, nor repeat cytology, has previously been shown to provide useful triage.²⁹ However, in this study, the combination of HPV + *TERC* testing showed the potential to provide effective triaging and would thus reduce the referral of LSIL cases for colposcopy. Furthermore, regarding the detection of CIN3⁺ cases, the combination of HPV + *TERC* testing appears to have few advantages compared with HPV test both in cytologic ASCUS and LSIL groups, and although the sensitivity resulting from the combination of these two methods increased significantly, the NPV only rose slightly and did not reach statistical significance.

There is a very high rate of spontaneous regression associated with CIN1, therefore management of CIN1 is recommended to include cytological follow-ups at defined intervals according to the 2006 consensus guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP).³⁰ However, not all cases of CIN1 will regress. It has been observed that a small number of cases will actually progress, and based on data of an ASCUS/LSIL Triage Study (ALTS), ~12% of women who have CIN1 or less have a risk of developing CIN2/3 within 2 years.³¹ Correspondingly, it is of great importance to distinguish transforming CIN1 lesions from naturally regressing CIN1 lesions. While some studies have reported that detection of *TERC* amplification could predict the progression of CIN1/2 cases to CIN3 with a predictive sensitivity of 100%,¹³ 428/2054 (20.8%) cases of CIN1 in this study exhibited *TERC* amplification. Additional evaluations are being performed to identify the patients of this group who are experiencing more aggressive biological behavior of their CIN1 cases, and then the predictive value of *TERC* amplification in relation to disease progression can be evaluated.

It is important to note, however, that this study enrolled a hospital-based population of high-risk women and was not representative of the general population. Therefore, while the incidence of CIN2⁺ was >40% in the 2316 patients whose data were used to calculate the NPV, this is much higher than the previously reported rate of 8%.³²⁻³⁴ This would explain the low NPV of HPV testing to detect CIN2⁺ (ie, 82.4%). Furthermore, the results of this study may be more applicable to high-risk women undergoing consultation rather than primary cervical screenings.

In summary, FISH can be performed on cervical exfoliated cells preserved in Thin-prep or Autocyte reserving fluid after the preparation of thin-layer cytopathologic slides without the need for additional collection of samples. As such, this would prevent the need to obtain additional samples for analysis. Detection of *TERC* amplification in cervical cells using the FISH technique was also shown to be a valuable method in combination with

cytology and/or HPV testing for the diagnosis of cervical disorders. The superiority of this approach was evidenced by the fact that *TERC* amplification detection was associated with higher sensitivity and specificity in distinguishing high-grade cervical lesions and invasive cancers from low-grade lesions in patients ranging in age from 18 to 65 years. Clinically, detection of *TERC* amplification has the potential to help distinguish between cases of CIN1 and CIN2, as well as to triage cytological ASCUS and LSIL patients to avoid unnecessary colposcopy procedures. However, additional prospective studies will be needed to confirm these results.

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