

Genomic amplification of HPV, h-TERC and c-MYC in liquid-based cytological specimens for screening of cervical intraepithelial neoplasia and cancer

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Abstract. Cervical cancer is one of the most prevalent female cancer types in developing countries. ThinPrep cytological test (TCT) and human papillomavirus (HPV) detection are canonical screening methods for cervical cancer currently. However, there are limitations to these techniques. The aim of the present study was to identify efficient and practical methods for the screening of cervical intraepithelial neoplasia (CIN) and carcinoma. Residual PreservCyt specimens were obtained from 1,000 women who were admitted between August 2013 and December 2015. TCT, human telomerase RNA component (h-TERC) fluorescent *in situ* hybridization (FISH), MYC-specific FISH and surface plasmon resonance (SPR)-HPV genotyping were performed, followed by histopathology for those patients with positive results in any of the four tests. As a result, 106, 64, 56 and 112 patients were positive in the TCT, h-TERC, c-MYC and SPR-HPV tests, respectively, resulting in 213 being scheduled for histopathology; inflammation was identified in 159 patients, CIN I in 31, CIN II in 14, CIN III in seven and invasive cervical cancer in two patients. Using histopathology as the gold standard, TCT exhibited the highest sensitivity (87.04%), while h-TERC analysis had the highest specificity (81.76%). Parallel tests demonstrated that the Youden's index of TCT + h-TERC was the highest (0.49), while the serial analysis reported that TCT + HPV had the highest Youden's index (0.53) compared with any of the biomarkers alone (TCT, 0.50; HPV, 0.29; h-TERC, 0.47). In conclusion, dual positive TCT and HPV may be an efficient approach for basic screening of cervical lesions. h-TERC amplification may serve as an auxiliary test to improve the specificity.

Introduction

Cervical cancer is one of the most prevalent female cancer types in developing countries, second only to breast cancer (1). In China, it is estimated that ~61,691 women are newly diagnosed with invasive cervical cancer (ICC) annually and 29,526 of these succumbed to this disease in 2012 (2). Shanxi province has been identified as a high-incidence area, with an incidence rate of 23.04 per 100,000 (3,4). Cervical cancer develops from the associated precursor lesion, cervical intraepithelial neoplasia (CIN). Thus, it is necessary to develop effective strategies for mass screening of CIN, in order to reduce the incidence of cervical cancer.

The ThinPrep cytological test (TCT) and human papillomavirus (HPV) detection are canonical screening methods for cervical cancer currently (5-7). However, there are limitations to these techniques. For example, TCT is only capable of identifying patients with abnormal cell morphology, and cannot determine if these patients are at high-risk of lesion progression. Additionally, the results of TCT may be influenced by sampling and slide quality, based on the skill and experience of the practitioner (8). It has also been reported that HPV infection is reversible in the reproductive system and only 1-2% of persistent HPV infections progress to neoplasia (9,10). Thus, it is beneficial to investigate novel biomarkers for the diagnosis of CIN and evaluation of the likelihood of lesion progression.

Recent studies have demonstrated that ICC invariably possesses extra copies of the chromosome arm 3q, which contains the human telomerase RNA component (h-TERC) gene in the 3q26 region (11,12). h-TERC encodes the template for telomerase RNA, corresponding to the repeat sequence which is added in tandem to the ends of chromosomes, in order to maintain the telomere length (13). Thus, abnormal amplification of h-TERC may lead to the cells having increased telomeres and enhanced proliferation ability, resulting in the formation of cervical tumors (12,13). Detection of h-TERC amplification may be beneficial for the diagnosis of CIN and ICC (14). c-MYC, located in chromosomal region 8q24, is reported to be the most common integration site in the HPV genome (15). The c-MYC gene may be overexpressed simultaneous to the amplification of HPV, which is associated with the acquisition of a malignant phenotype in cervical cells (16). Therefore, c-MYC may also

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be an important oncogene involved in tumor progression, and hence may be a potential biomarker for cervical cancer (17). Previously, there have been studies to evaluate the diagnostic value of h-TERC (18) or c-MYC (19) alone in TCT and HPV, or a combination of h-TERC and c-MYC (11) in CIN and cancer. However, limited studies have been performed in order to determine which is the most efficient and practical method based on the four tests (TCT, HPV DNA, h-TERC and c-MYC) (8). Additionally, the majority of studies are aimed at demonstrating the sensitivity and specificity of the aforementioned biomarkers in distinguishing high-grade cervical lesions (>CIN2⁺ or ≥CIN2) and invasive cancer types from low-grade lesions (11,18,19); consequently, studies rarely focus on the difference between normal and all precursor lesions as well as ICC. Furthermore, the conclusions remain inconsistent between different studies. For example, Zheng *et al* (18) observed that among three methods, h-TERC testing displayed the highest specificity and positive predictive value, and HPV testing displayed the highest sensitivity, while TCT was not optimal for any of the diagnostic parameters. Jiang *et al* (20) demonstrated that for the detection of advanced cervical lesions, cytological evaluations were optimal (93.3%) in terms of specificity, followed by h-TERC amplification (83.8%) and HPV DNA analyses (39.3%). Zhao *et al* (19) reported that the specificity of fluorescence *in situ* hybridization (FISH) analysis with a c-MYC-specific probe seemed to be higher compared with TCT and HPV DNA testing; however, this was not consistent with the results from Gao *et al* (8) or Li *et al* (11). The diagnostic performance of c-MYC [area under the curve (AUC)=0.865] was slightly superior to that of h-TERC (AUC=0.843) in the study performed by Gao *et al* (8), although contrasting results were obtained from Li *et al* (11) (c-MYC AUC=0.799 vs. h-TERC AUC=0.838). Therefore, further studies are required in order to confirm the diagnostic abilities of h-TERC and c-MYC compared with TCT and HPV DNA testing.

The aim of the present study was to determine the optimal programs for screening precancerous lesions and cervical carcinoma among women in Shanghai, one of the largest cities in China (21), by TCT, h-TERC- and MYC-specific FISH and surface plasmon resonance (SPR)-HPV genotyping.

Materials and methods

Patients and specimens. Residual liquid-based cytology PreservCyt specimens were obtained from 1,000 women (aged 20-68 years) who were admitted to the gynecological clinic of Ren Ji Hospital of Shanghai Jiao Tong University (Shanghai, China) for routine screening between August 2013 and December 2015. None of the patients had received cytological tests within 6 months prior to cervical therapy, including radical surgery (hysterectomy and bilateral pelvic lymphadenectomy) with or without adjuvant radiotherapy or chemotherapy. Furthermore, none of the patients had additional neoplastic diseases. All specimens underwent HPV DNA, h-TERC, c-MYC and TCT testing. Histopathological examination was performed for patients who received positive results for any of the aforementioned tests. The Ethics Committee of Ren Ji Hospital of Shanghai Jiao Tong University approved this study. Written informed consent was obtained from all patients when the biopsy was performed.

Cytological examination. The liquid-based specimens were automatically processed with the Cytoc T2000 ThinPrep® system (Cytoc Corp., Marlborough, MA, USA) and stained with the Papanicolaou stain method [95% ethyl alcohol fixation for 10 min; Harris hematoxylin (Papanicolaou solution A; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 3 min; running water, 2-3 times; 0.05% hydrochloric acid, 30 sec; 95% ethyl alcohol rinsing 2-3 times; orange G (Papanicolaou solution B, Sigma-Aldrich; Merck KGaA; 1 drop for 10 sec); 95% ethyl alcohol for 30 sec; 100% ethyl alcohol for 30 sec; polychromatic solution EA50 for 3 min; 95% ethyl alcohol for 2 min; 100% ethyl alcohol for 2 min; xylene for 2 min; all at room temperature; and mounted in a mounting medium (Permount; Thermo Fisher Scientific, Inc., Waltham, MA, USA)]. Cytological diagnoses were classified independently by two cytopathology physicians, under double-blinded conditions according to the 2001 Bethesda System (22). Diagnoses were as follows: i) Negative for intraepithelial lesion or malignancy; ii) epithelial cell abnormality e.g. atypical squamous cells of undetermined significance (ASCUS); iii) atypical squamous cells in which high-grade squamous intraepithelial lesion cannot be excluded (ASCH); iv) low-grade squamous intraepithelial lesion (LSIL); v) high-grade squamous intraepithelial lesion (HSIL); vi) squamous cell carcinoma; and vii) atypical glandular cells.

h-TERC and c-MYC gene detection. FISH was used in order to detect the amplification of h-TERC and c-MYC genes as previously described (8), according to the following procedures.

Preparation of slides. ThinPrep samples were enzymatically digested in 0.1% collagenase (type B; 20-30 min), centrifuged (2,000 x g at 37°C for 10 min), re-suspended in deionized water (20 min) and fixed twice with a methanol + acetic acid mixture (volume ratio, 3:1; 10 min) to prepare one-layer slides.

Pretreatment. Following rinsing twice with 2X sodium chloride plus sodium citrate (SSC; 5 min), the slides were placed in 0.1 M HCl for 5 min and incubated with a pepsin solution (0.05% pepsin/0.01 M HCl; (Sigma-Aldrich; Merck KGaA) for 10 min. Thereafter, the samples were subjected to dehydration through a graded ethanol series (70, 85 and 100%).

FISH. The prepared samples and probe mixture (consisting of probe, 2 ml; hybridization buffer, 7 ml; and deionized water, 1 ml) were denatured in 70% formamide/2X SSC for 5 min and dehydrated with the above graded ethanol series for 3 min each. Dual-color fluorescence probes were used for the detection of h-TERC [red, gene locus-specific probe (GLP) h-TERC probe; green, GLP chromosome 3 centromere-specific (CSP3) control probe], while the single probe was performed for measurement of c-MYC (red) (GP Medical Technologies, Beijing, China). The denatured probes were added onto the denatured slides and hybridized overnight at 42°C. Following removal of the cover slips, the slides were rinsed with 0.3% NP-40/0.4X SSC for 2 min at 67°C, followed by washing with 0.1% NP-40/2X SSC and 70% ethanol for 30 sec and 3 min, respectively. The slides were mounted with DAPI at 37°C for 10-20 min to counterstain.

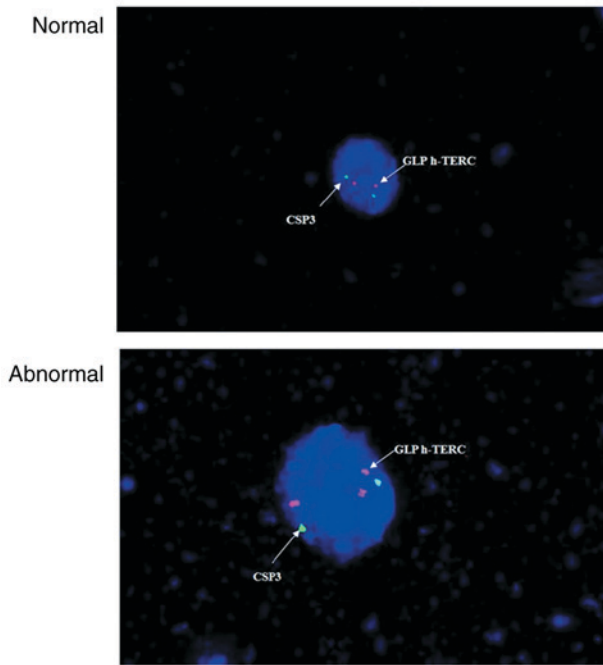


Figure 1. Normal vs. abnormal h-TERC signal from fluorescence *in situ* hybridization. CSP, chromosome 3 centromere-specific; h-TERC, human telomerase RNA component; GLP, gene locus-specific probe.

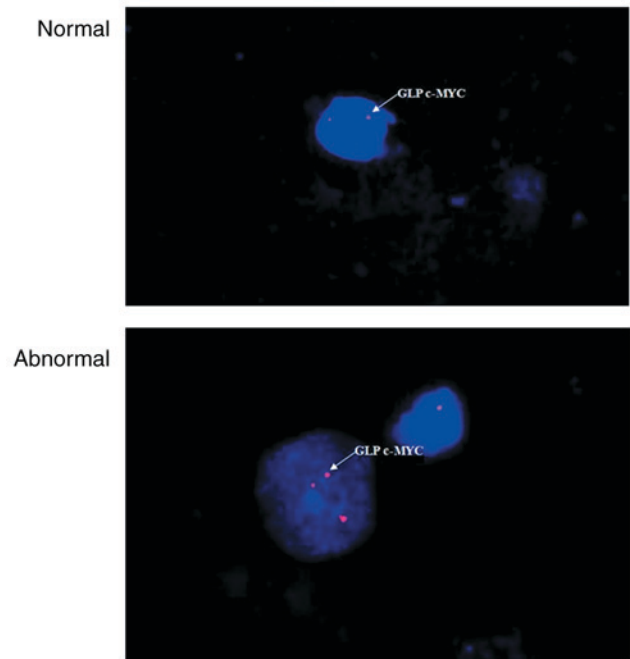


Figure 2. Normal vs. abnormal c-MYC signal from fluorescence *in situ* hybridization. CSP, chromosome 3 centromere-specific; GLP, gene locus-specific probe.

FISH signal interpretation. Images of the slides were obtained on a fluorescence microscope (DM 2500; Leica Microsystems GmbH, Wetzlar, Germany) and analyzed using VideoTest-FISH software (version 2.0; VideoTest, St. Petersburg, Russia). The cell areas were determined through a DAPI filter (x100 objective). A nucleus with >2h-TERC/CSP3 signals (Fig. 1) or >2c-MYC signals (Fig. 2) was scored positive, while all remaining cells were considered normal.

HPV DNA detection and genotyping. SPR-based tests conducted at the Beijing Jinpujia Medical Technology Co., Ltd. (Beijing, China) were used for HPV DNA detection and genotyping (23,24). Briefly, genomic DNA in ThinPrep cell suspension samples was isolated using Fast Extract Solution and amplified on an Eppendorf Mastercycler polymerase chain reaction (PCR) machine (model no. 5333; Eppendorf, Hamburg, Germany) with Taq polymerase (cat. no. R001A; Takara, Inc., Otsu, Japan) using the following procedure: 2 min at 50°C, 4 min at 94°C; 28 cycles of 30 sec at 94°C, 45 sec at 48°C, and 20 sec at 72°C; followed by 25 cycles of 30 sec at 94°C, 45 sec at 65°C and 20 sec at 72°C. The HPV genotyping of the amplified DNA product was performed using an SPR biosensor chip reading instrument (W2600; Beijing Jinpujia Medical Technology Co., Ltd., Beijing, China) which is able to detect 16 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 81) and eight low-risk (6, 11, 40, 42, 43, 44, 54 and 70) HPV subtypes simultaneously. The primers were provided by the Beijing Jinpujia Medical Technology Co., Ltd. and not shown.

Histopathological evaluation. A cell block was prepared from the ThinPrep samples by centrifugation at 1,500 x g for 10 min at 4°C and fixed in 10% formaldehyde overnight at 4°C. Subsequently, the specimen were transferred to graded

ethanol for dehydration (70, 80, 90, 95 and 100%; each for 3 min), embedded in 5- μ m-thick paraffin and stained with hematoxylin (5 min) and eosin (5 min) at room temperature for the pathological examination. The diagnosis was divided into four categories: i) Normal/inflammation; ii) CIN I (mild atypical hyperplasia and cellular atypism); iii) CIN II (moderate atypical hyperplasia and distinct cellular atypism); and iv) CIN III (severe atypical hyperplasia and significant cellular atypism) and ICC.

Statistical analysis. All the statistical analyses were performed with SPSS software (version 18.0 for Windows; SPSS Inc., Chicago, IL, USA). The enumerative results were expressed as n (%) and compared using a χ^2 test (or Fisher's exact test). Using the histopathological diagnosis as the gold standard, the sensitivity [a/(a+c) x100%], specificity [d/(b+d) x100%], positive predictive value [a/(a+b) x100%], negative predictive value [d/(c+d) x100%], misdiagnosis rate [b/(b+d) x100%], omission diagnosis rate [c/(a+c) x100%] and Youden's index (Y=sensitivity + specificity-1.0) of the four screening programs for identification of CIN and ICC were calculated, where 'a' referred to true positive, 'b' was the false positive, 'c' was the false negative, and 'd' was true negative. Combined diagnosis was evaluated in parallel (if any of the methods were positive) or series (if all the methods were positive). P<0.05 was considered to indicate a statistically significant difference.

Results

Correlation between histopathological findings and TCT, h-TERC, c-MYC and HPV testing. Of the 1,000 cytological cases, 106 were reported to be positive in the TCT test, including ASCUS in 57 patients, ASCH in 13 patients, LSIL in 30 patients and HSIL in six patients. FISH analysis revealed

that 64 patients (6.40%) were h-TERC positive and 56 (5.60%) were c-MYC positive. SPR-HPV genotyping demonstrated that 112 patients (11.20%) were HPV positive, of which 75 were infected with high-risk types, 28 were infected with low-risk types and nine were combined high- and low-risk types. In addition, infection with a single HPV subtype was observed in 66 patients (58.93%) and infection with multiple HPV subtypes was reported in 46 patients (41.07%).

Following screening, 213 patients were scheduled for histopathological examination due to a positive result in any of the aforementioned tests. The results reported inflammation in 159 patients, CIN I in 31 patients, CIN II in 14 patients, CIN III in 7 patients and ICC in two patients.

According to the histopathological findings, the rate of h-TERC, c-MYC expression and high-risk HPV infection [with the dominant genotypes of HPV16 (75.0%, 21/28), HPV18 (72.7%, 8/11), HPV58 (57.1%, 8/14), HPV52 (25.0%, 2/8) and HPV33 (20.0%, 1/5) (data not shown)], increased gradually with the increase in the severity of lesions ($P < 0.05$; Table I). However, no significant differences in ASCH and HSIL of cytological examination were observed among different histopathological diagnoses. The LSIL ratio in the cytological examination was significantly elevated, but ASCUS ratio was significantly declined with the increase in the severity of lesions ($P < 0.05$; Table I).

Diagnostic value of each method for cervical lesions. Using the pathological result of 'normal' as the criterion, the sensitivity, specificity, positive predictive value, negative predictive value, misdiagnosis rate, omission diagnosis rate and Youden's index of TCT, HPV, h-TERC and c-MYC for the detection of cervical lesions, including CIN and ICC, were determined. The results demonstrated that TCT exhibited the highest sensitivity (87.04%) and lowest omission diagnosis rate (12.96%), while h-TERC gene analysis had the highest specificity (81.76%) and the lowest misdiagnosis rate (18.24%), suggesting these two methods may be important when screening cervical lesions (Table II). These results were confirmed with the Youden's index, with a score of 0.5 and 0.47 for TCT and h-TERC, respectively. Although no significant difference in the sensitivity was observed between TCT and HPV, as well as between h-TERC and c-MYC ($P > 0.05$), Youden's indices of HPV and c-MYC were approximately 0.2, suggesting their poor utility for case detection (Table II).

Furthermore, the combined diagnostic values of the above tests were analyzed. The results indicated that the sensitivity increased from 87.04 to $>90\%$ when the TCT was combined with the h-TERC, c-MYC and/or HPV test (any positive; Table III), however the specificity decreased from 62.89 to $<50\%$, with the highest specificity observed in combined h-TERC and c-MYC testing (62.89%). Nevertheless, the Youden's index of TCT and h-TERC remained relatively high (0.49), further implying their importance.

In addition, serial tests (both or all positive) were also performed. As a result, the specificity of any combination was notably higher compared with the parallel test, yet the sensitivity was decreased, with the highest sensitivity being 62.96%. According to the Youden's index, TCT + HPV (0.53) may be the most effective for diagnosis of cervical lesions, followed by TCT + h-TERC (0.47) and h-TERC + HPV (0.47) (Table III). Comprehensively, dual positive TCT and

Table I. Cytological, positive h-TERC, c-MYC expression and HPV infection findings according to histopathological diagnosis.

Histopathological classification	No. patients	TCT, n (%)				h-TERC positive, n (%)			c-MYC positive, n (%)			HPV positive, n (%)		
		ASCUS	ASCH	LSIL	HSIL	LSIL	HSIL	High risk	Low risk	Combined	High risk	Low risk	Combined	
Normal	159	46 (28.93)	1 (0.63)	12 (7.55)	0 (0.00)	29 (18.24)	32 (20.13)	41 (25.79)	25 (15.72)	6 (3.77)				
CIN I	31	10 (32.26)	7 (22.58)	8 (25.81)	0 (0.00)	18 (58.06)	11 (35.48)	16 (51.61)	2 (6.45)	3 (9.68)				
CIN II	14	1 (7.14)	5 (35.71)	6 (42.86)	1 (7.14)	9 (64.29)	7 (50.00)	10 (71.43)	1 (7.14)	0 (0.00)				
CIN III	7	0 (0.00)	0 (0.00)	3 (42.86)	4 (57.14)	6 (85.71)	4 (57.14)	6 (85.71)	0 (0.00)	0 (0.00)				
ICC	2	0 (0.00)	0 (0.00)	1 (50.00)	1 (50.00)	2 (100.00)	2 (100.00)	2 (100.00)	0 (0.00)	0 (0.00)				
P-value	-	<0.001	0.076	0.003	0.342	0.01	<0.001	0.02	0.52	0.552				

CIN, cervical intraepithelial neoplasia; ICC, invasive cervical cancer; ASCUS, atypical squamous cell of undetermined significance; ASCH, atypical squamous cells in which high-grade squamous intraepithelial lesion cannot be excluded; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; TCT, ThinPrep cytological test; HPV, human papillomavirus; h-TERC, human telomerase RNA component.

Table II. Diagnostic value of TCT, h-TERC, c-MYC and HPV for cervical lesions.

Test	Results	CIN and ICC		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Misdiagnosis rate (%)	Omission diagnosis rate (%)	Youden's index
		Normal	CIN and ICC							
TCT	+	47	59	87.04	62.89	44.34	93.46	37.11	12.96	0.50
	-	7	100	-	-	-	-	-	-	-
h-TERC	+	35	29	64.81 ^a	81.76 ^a	54.69	87.25	18.24 ^a	35.19 ^a	0.47
	-	19	130	-	-	-	-	-	-	-
c-MYC	+	24	32	44.44 ^a	79.87 ^a	42.86	80.89 ^a	20.13 ^a	55.56 ^a	0.24
	-	30	127	-	-	-	-	-	-	-
HPV	+	40	72	74.07 ^c	54.72 ^{b,c}	35.71 ^b	86.14	45.28 ^{b,c}	25.93 ^{a,c}	0.29
	-	14	87	-	-	-	-	-	-	-

^aP<0.05 vs. TCT; ^bP<0.05 vs. h-TERC; ^cP<0.05 vs. c-MYC. TCT, ThinPrep cytological test; HPV, human papillomavirus; h-TERC, human telomerase RNA component; CIN, cervical intraepithelial neoplasia; ICC, invasive cervical cancer.

HPV were suggested to be an efficient approach for the basic screening of cervical lesions. h-TERC amplification may serve as an auxiliary test for TCT and HPV testing, to improve the specificity. This conclusion may be credible due to the highest Youden's index in TCT + HPV + h-TERC (0.37) among the three combination groups (TCT + h-TERC + c-MYC, 0.25; TCT + c-MYC + HPV, 0.27; h-TERC + c-MYC + HPV, 0.23).

Discussion

In the present study, for the first time to the best of our knowledge, the optimal programs for screening CIN and cervical carcinoma among women in Shanghai were demonstrated (21), through simultaneous detection of TCT, h-TERC, MYC and HPV. The results demonstrated that although TCT (87.04%) and HPV testing (74.07%) alone had higher sensitivity for screening CIN and cervical carcinoma, their specificities (62.89 and 54.72%, respectively) were significantly lower compared with the alternative tests analyzed, suggesting that there may be limitations when using these tests alone to screen precancerous cervical lesions. This trend seemed to be in accordance with previous studies (19,25); however, the quantitative values were slightly different, which may be attributed to the differences in the sample size (25), regional location of the population (21) and detection methods (18,19). In particular, it has been reported that there are discordant results between SPR and hybrid capture II (HC2) tests or direct DNA sequencing assays (23,24). Nevertheless, the SPR test has the incomparable advantages of a low-cost, rapid detection and an easy-to-use method with the potential for automation (23,24). In addition, the SPR method is able to test 24 HPV subtypes, which is higher compared with the 13 subtypes which are currently able to be tested using the HC2 test (21), or the 21 subtypes which are able to be detected via the CAPE HPV genotyping assay kit (19). The results from the present study suggested that the SPR test may be a superior approach for HPV genotyping in a clinical setting. When the TCT and HPV tests were combined and one or both of the markers amplified to be positive, the sensitivity and the specificity were increased to 98.15 and 89.94%, respectively, displaying the highest Youden's index values (0.53) compared with all alternative combinations. These results implied that the combination of the TCT and HPV tests may improve the sensitivity of cervical disease screening and reduce the rate of misdiagnosis, leading to the timely detection of this disease in high-risk populations. These results further demonstrated the necessity of detection of TCT and HPV in the clinic as previously reported (25,26).

Although combined TCT and HPV tests may improve the sensitivity and the specificity to 98.15 and 89.94% respectively, a number of patients may still be misdiagnosed and missed. Therefore, alternative biomarkers are required to serve as supplementary detection methods. The present study reported that h-TERC gene analysis had the highest specificity (81.76%) and the lowest misdiagnosis rate (18.24%). Additionally, the combination of TCT, HPV and h-TERC displayed 100% sensitivity and 98.11% specificity, suggesting h-TERC may be the principal gene in cervical carcinogenesis, which is in line with results from previous studies (19,27). It has been reported that the h-TERC gene is significantly upregulated in

Table III. Parallel and serial tests of combined TCT, h-TERC, c-MYC and HPV testing for diagnosis of cervical lesions.

A, Parallel							
Method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Misdiagnosis rate (%)	Omission diagnosis rate (%)	Youden's index
TCT + h-TERC	100.00	49.06	40.00	100.00	50.94	0.00	0.49
TCT + c-MYC	94.44	46.54	37.50	96.10	53.46	5.56	0.41
TCT + HPV	98.15	27.67	31.55	97.78	72.33	1.85	0.26
h-TERC + c-MYC	75.93	62.89	41.00	88.50	37.11	24.07	0.39
h-TERC + HPV	88.89	38.99	33.10	91.18	61.01	11.11	0.28
c-MYC + HPV	85.19	37.11	31.51	88.06	62.89	14.81	0.22
TCT + h-TERC + c-MYC	100.00	33.33	33.75	100.00	66.67	0.00	0.33
TCT + h-TERC + HPV	100.00	14.47	28.42	100.00	85.53	0.00	0.14
TCT + c-MYC + HPV	98.15	14.47	28.04	95.83	85.53	1.85	0.13
h-TERC + c-MYC + HPV	90.74	21.38	28.16	87.18	78.62	9.26	0.12
B, Serial							
Method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Misdiagnosis rate (%)	Omission diagnosis rate (%)	Youden's index
TCT + h-TERC	51.85	95.60	80.00	85.39	4.40	48.15	0.47
TCT + c-MYC	37.04	96.23	76.92	81.82	3.77	62.96	0.33
TCT + HPV	62.96	89.94	68.00	87.73	10.06	37.04	0.53
h-TERC + c-MYC	33.33	98.74	90.00	81.35	1.26	66.67	0.32
h-TERC + HPV	50.00	97.48	87.10	85.16	2.52	50.00	0.47
c-MYC + HPV	33.33	97.48	81.82	81.15	2.52	66.67	0.31
TCT + h-TERC + c-MYC	25.93	99.37	93.33	79.80	0.63	74.07	0.25
TCT + h-TERC + HPV	38.89	98.11	87.50	82.54	1.89	61.11	0.37
TCT + c-MYC + HPV	27.78	99.37	93.75	80.20	0.63	72.22	0.27
h-TERC + c-MYC + HPV	24.07	98.74	86.67	79.29	1.26	75.93	0.23

TCT, ThinPrep cytological test; HPV, human papillomavirus; h-TERC, human telomerase RNA component.

numerous cancer types (28,29). Inhibition of h-TERC blocks tumor growth and promotes cell apoptosis, including cervical cancer (30). This phenomenon was also observed in the present study, with the h-TERC-positive rate significantly increased from the histological diagnoses of normal (18.24%), CIN I (58.06%), CIN II (64.29%), CIN III (85.71%) to ICC (100%). These results indicate that h-TERC amplification may be a useful genetic testing marker that might assist in the clinical histopathological analysis for the differential diagnosis of normal or CIN cases, and may even distinguish between low-grade (<CIN I) and high-grade (>CIN II) cervical lesions.

Furthermore, extensive studies have demonstrated that the transcription factor c-MYC may serve an important role in carcinogenesis, due to its effect on basic cell processes including cell proliferation, the cell cycle and apoptosis (31-33). Thus, c-MYC amplification may also be a potential diagnostic indicator for cervical cancer (8,11,19). In the present study, it was observed that the c-MYC-positive rates increased with increasing severity of histological diagnosis. However, the

fact that Youden's index of c-MYC itself was lower and the addition of c-MYC did not increase the Youden's index for the alternative methods, suggests that c-MYC may be not the most ideal biomarker in the screening of cervical lesions.

There are a number of limitations to the present study. First, the study was a single center study and the sample size was relatively small, which may have led to the underestimation or overestimation of the diagnostic values of the biomarkers. Second, the aim of the study was to demonstrate the diagnostic performance of the biomarkers for differentiating CIN and ICC from healthy controls; therefore, further investigations are required in order to validate the utility of the biomarkers for screening different cervical lesion severities (11). Third, beyond h-TERC and c-MYC genes, numerous studies have reported that the epigenetically-regulated genes, including paired box 1 (34) and zinc finger protein 582 (35), may serve as genetic biomarkers for detecting high-grade CIN lesions and cervical cancer. Therefore, it may be beneficial for further studies to also consider these epigenetic factors.

In conclusion, the results of this preliminary study revealed that a combination of TCT and HPV testing may be the most efficient approach for the basic screening of cervical lesions in Shanghai. h-TERC amplification may serve as an auxiliary test to improve the specificity. However, further investigation with larger samples collected from a multicenter study is required in order to confirm these results.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Author's contributions

WJ and WD participated in the design of this study. WL and ZH were involved in the sample collection. WJ and LQ served important roles in statistical analyses. WJ, WL and WD contributed to the acquisition and interpretation of data. WJ and WD drafted and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Ren Ji Hospital of Shanghai Jiao Tong University approved this study. Written informed consent was obtained from all patients when the biopsy was performed.

Patient consent for publication

All patients agreed with their data used for publication purposes.

Competing interests

The authors declare that they have no competing interests.

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