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ORIGINAL RESEARCH

The Application of Liquid-Based Cytological Detection for P16, Cytologic Evaluation and High-Risk Human Papillomavirus Testing in Cervical Cancer Screening: A Clinical Evaluation

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Objective: The aim of this study was to clinically evaluate the application of liquid-based cytology P16, cytologic evaluation, and high-risk human papillomavirus (HR-HPV) testing in cervical cancer screening.

Methods: This study screened 900 women, who attended the outpatient clinic, according to the exclusion criteria of study participants. The study participants' screening results of liquid-based cytology P16, cytologic evaluation, and HR-HPV testing were analyzed. According to the pathological results of the biopsy, the efficacy of different screening strategies for the identification of high-grade lesions was evaluated.

Results: The positive rate of p16 expression increased with the severity of cervical lesions. P16 had the highest sensitivity and negative predictive value in identifying high-grade lesions (98.45% and 99.67%, respectively). Liquid-based Papanicolaou test (LBP), on the other hand, had the lowest sensitivity (85.27%) but the highest specificity (85.88%). HR-HPV's positive predictive value and accuracy rate were the lowest (32.77% and 70.03%, respectively). The difference was statistically significant ($P < 0.05$). Dual combinations of certain tests were set up for this study; P16+LBP, HPV+LBP, and P16+HPV had sensitivities of 98.45%, 96.90%, and 99.22%, and specificities of 80.29%, 63.42%, and 64.33%, respectively. The P16 screening rates of histological and liquid-based cytology approaches were 75.74%.

Conclusion: Compared with traditional LBP+HPV, the application of a test that solely screen for P16 or the combined screening method that involves the screening of P16 is more effective in identifying high-grade lesions.

Keywords: biomarkers, liquid-based cytology, p16, HR-HPV, screening of high-grade cervical lesions

Background

Cervical cancer, which is caused by multiple factors such as persistent high-risk human papillomavirus (HR-HPV) infection, is a common malignant tumor arising from the female reproductive tract. Cervical cancer has a high incidence rate, second only to breast cancer, and poses a serious threat worldwide to female reproductive health.[1] The onset of invasive cervical cancer is preceded by a long period of precancerous lesions. Therefore, efficient screening of high-risk groups for cervical cancer and accurate screening for high-grade precancerous lesions is the key to reducing cervical cancer mortality.[2] The earliest screening method for cervical cancer is liquid-based cytology, ie, the liquid-based Papanicolaou (LBP) test. However, the results of LBP are limited by the quality of specimen preparation and the varying degrees of technical expertise of the cytopathologists, all of which may result in poor reproducibility and low sensitivity. However, HR-HPV testing is objective, convenient, automated, and sensitive[3] and has a good negative predictive value. HPV-negative people have a low probability of developing cervical cancer.[3] Therefore, the cervical cancer screening method has gradually shifted in many parts of the world from the application

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of cytology to HR-HPV screening as the primary screening,[4] or the primary cytology screening for HPV + detection triage involving HPV, the combination of cytology and HPV screening, and the primary HPV screening + cytology triage.[5,6] However, the high sensitivity of HR-HPV and the inability to identify persistent infections have contributed to too many referrals for colposcopy and overtreatment due to the fear of cervical cancer.[7] In recent years, tumor suppressor P16 has been regarded as a biological marker of early cervical cancer owing to its high sensitivity and specificity related to high-risk HPV infection, precancerous lesions, and cervical cancer.[8] Routine histopathological diagnosis is the "gold standard" method.

Nevertheless, histological detection of P16 in biopsy specimens requires invasive procedures after screening and referral to colposcopy, and thus, it cannot be used as a screening method.[9] This study used a new liquid-based cytology detection method for P16, protected under the Chinese independent intellectual property rights, to perform immunocytochemical staining of P16 protein on the remaining specimens of cervical cancer after liquid-based cytology screening. The method was also compared with the P16 screening results of colposcopy tissues to determine the diagnostic value of liquid-based cytological detection for P16 in high-grade lesions and the application value of different testing modes combined with HR-HPV or liquid-based cytological detection for P16 in the screening of cervical cancer and precancerous lesions to optimize the initial screening and triage strategy.

We aim to reduce the number of colposcopy examinations as much as possible after weighing the methods' risks and benefits and identifying more target groups with precancerous cervical lesions.

Materials and Methods

Study Participants

From January 2018 to July 2019, patients aged 21–69 years who attended the cervical cancer screening were recruited from the outpatient clinic of the Department of Obstetrics and Gynecology, Peking University First Hospital. A total of 900 patients who met these criteria were enrolled. All participants provided informed consent, in accordance with the Declaration of Helsinki.[1] The exclusion criteria of study participants are as follows: (i) pregnant women, (ii) lactating women, (iii) patients with a history of HPV treatment, (iv) patients with a history of cervical lesions or cervical cancer surgery, (v) patients with a history of radiotherapy and chemotherapy, (vi) women with incomplete uterus or cervix, (vii) patients with condyloma acuminatum or serious medical and surgical diseases. The specimens collected from all patients were subjected to the cytology of exfoliated cervical cells (LBP), liquid-based cytological detection for P16, and HR-HPV test. The patients were instructed not to engage in copulation, apply medication to the vagina, and have the vaginal examination and surgical operation within 72 h. All the enrolled study participants were required to fill out a pathological examination sheet and evaluated by gynecologists after the gynecological examination. The specimens were collected by specimen brush. This study was performed with the approval of the ethics committee of Peking University First Hospital.

Liquid-Based Cytology (LBP)

The preparation and staining procedures were completed using the SurePath Liquid-Based Cytology System (BD Diagnostics, Tripath, Burlington, NC, US), and the interpretation was performed using the TBS standard, the new TBS reporting method revised in 2014.[10] LBP-positive cases refer to cytological abnormalities, including squamous cell-related and glandular cell-related abnormalities. Squamous cell abnormalities are further divided into atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells, and atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesions. (LSIL), high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC). Glandular abnormalities include atypical glandular cells (AGC), cervical adenocarcinoma in situ (AIS), and adenocarcinoma (AC).

Liquid-Based Cytological Detection for P16

Liquid-based cytological detection for P16 was performed on the remaining samples after LBP using the automatic immune cell stainer (JY-6000) and immunocytochemical method-based P16 antibody detection kit manufactured by Jiangyuan Medical (Guangzhou, China). The experiments were completed according to the test procedures. A conventional light microscope was used to view the cells and determine whether the cytoplasm or nucleus was

stained. If the cytoplasm (or accompanying nuclei) of epithelial cells was stained light brown on each smear, the specimen was positive; if they were not stained light brown, it was negative. The LBP and P16 tests are cytological methods that require a qualified pathologist to interpret the results. All LBP and P16 tests were completed by the same experienced cytologist to reduce the error of perception.

HR-HPV Testing

In this experiment, HPV typing and detection kit and Flow-through Hybridization HybriMax instrument (HHM-2 model) manufactured by Hybribrio (Chaozhou, China) were used, HybriMax technology was used to detect 21 HPV subtypes, ie, 15 common high-risk subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and 6 low-risk subtypes (6, 11, 42, 43, 44, cp8304). A positive test result for one or more of the 15 high-risk types indicated a positive HR-HPV test. The test was considered negative if all 15 high-risk subtypes were tested negative.

Indications for Colposcopy and Biopsy

The colposcopy is indicated if either one of the following conditions is fulfilled: (i) negative LBP test result but positive HPV16 and/or HPV18 test result; (ii) positive ASC-US result after LBP test with positive HR-HPV test result; (iii) non-ASC-US LBP test result accompanied by other abnormal cytology results with negative HR-HPV test result; (iv) observation of any abnormal proliferative or asymmetric asymmetry of the cervical surface during gynecological examination. Biopsies should be taken from patients with abnormal colposcopy results; some patients without lesions should be biopsied from areas with rich blood circulation, including endocervical curettage (ECC); this is subject to a specific situation.

Histopathological Diagnosis

Histopathological specimens were prepared by the pathology department of our hospital according to the workflow. Routine hematoxylin and eosin staining and immunohistochemical staining of P16 and Ki67 were performed on the tissue of each specimen using anti-P16 mouse monoclonal clones provided by Roche (Shanghai, China) and anti-Ki67 mouse monoclonal antibody provided by Dako. A histopathological examination was performed by two senior pathologists. They interpreted inflammation and grade I, grade 2, grade 3, and invasive cancer of cervical intraepithelial neoplasia as "gold standard" by histological findings. The diagnosis was made using the standard diagnostic terminologies and criteria outlined in the fourth edition (2014) of the WHO Classification of Female Reproductive Organs tumors.[11]

Statistical Analysis

The number of cases and percentages were used for general statistical description, and the sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and Youden index were used to analyze the screening efficacy of P16, LBP, and HR-HPV testing. The Chi-squared (χ^2) test was used to compare the sensitivity and specificity of the combined screening method of P16+LBP, LBP+HR-HPV, and HR-HPV+P16 to identify high-grade lesions and to find out whether there was a statistically significant difference between the negative and positive predictive values. A difference with $P < 0.05$ was considered statistically significant.

Results

Basic Characteristics of the Study Participants

A total of 900 valid cases were selected and recruited based on the inclusion and exclusion criteria. The study participants were aged 21 to 69 years (mean = 40.57 ± 10.77 years). There were 218 cases with a cytological diagnosis of ASCUS. The positive rate of P16 was statistically different among the cytology techniques ($\chi^2 = 423.0516$, $P < 0.0001$). The positive rate of P16 showed an upward trend from normal to HSIL classification, which is reflective of an increase in diagnostic grades in cytology (Table 1). Among the cases with normal cytopathology, the positive rates of P16 and HSIL were 8.94% and 98.55%, respectively. In different histopathological results, the positive rate of P16 was different ($\chi^2 = 571.5977$, $P < 0.0001$). As shown in Table 2, it can be seen that with the aggravation of cervical lesions, the positive rate of P16 tends to increase.

Table 1 Expression of P16 in Different Cytological Techniques

LBC	P16-Positive		P1-Negative	
	n	%	n	%
Normal	61	8.94	621	91.06
ASCUS	29	39.19	45	60.81
LSIL	17	50.00	17	50.00
ASC-H	33	94.29	2	5.71
HSIL	68	98.55	1	1.45
AGC-NOS/FN	4	66.67	2	33.33
Total	212	23.56	688	76.44

Note: χ^2 = 423.0516, P < 0.0001.
Abbreviations: ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; ASC-H, high-grade squamous intraepithelial lesions that cannot be excluded; HSIL, high-grade squamous intraepithelial lesions; AGC-NOS/FN, SARS type glandular cells or unclassified.

Table 2 Histopathological Distribution of P16 Screened by Different Cytological Techniques

Screening Method		Total		Histopathological Result											
				Normal		Inflammation/ Polyps/Condyloma		CINI		CINII		CINIII		Cancer	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
LBC	Normal	682	100.00	605	88.71	50	7.33	8	1.17	10	1.47	7	1.03	2	0.29
	ASCUS	74	100.00	42	56.76	14	18.92	6	8.11	4	5.41	8	10.81	0	0.00
	LSIL	34	100.00	15	44.12	4	11.76	4	11.76	7	20.59	4	11.76	0	0.00
	ASC-H	35	100.00	3	8.57	5	14.29	3	8.57	10	28.57	13	37.14	1	2.86
	HSIL	69	100.00	2	2.90	2	2.90	4	5.80	21	30.43	39	56.52	1	1.45
	AGC-NOS/FN	6	100.00	0	0.00	4	66.67	0	0.00	1	16.67	0	0.00	1	16.67
P16	Positive	212	100.00	31	14.62	41	19.34	15	7.08	52	24.53	68	32.08	5	2.36
	Negative	688	100.00	636	92.44	38	5.52	10	1.45	1	0.15	3	0.44	0	0.00
Hpv	Positive	356	100.00	168	47.19	50	14.04	21	5.90	47	13.20	68	19.10	2	0.56
	Negative	544	100.00	499	91.73	29	5.33	4	0.74	6	1.10	3	0.55	3	0.55
Total		900	100.00	667	74.11	79	8.78	25	2.78	53	5.89	71	7.89	5	0.56

Notes: Mantel-Haenszel χ^2 value for LBC & Histopathology = 474.1237, P < 0.0001; χ^2 value for P16 & Histopathology = 571.5977, P < 0.0001.
Abbreviations: CIN I, cervical intraepithelial degeneration grade I; CIN II, cervical intraepithelial degeneration grade II; CINIII, cervical intraepithelial grade III.

Efficacy of P16, HPV, and LBP Testing

According to Table 3 for comparison, the sensitivity and negative predictive value of P16 for detecting cervical HSIL$^+$ lesions were the highest (98.45% and 99.67%, respectively). LBP had the lowest sensitivity (85.27%) but the highest specificity (85.88%). HR-HPV had the highest positive predictive value and lowest accuracy rate (32.77% and 70.03%, respectively).

Comparison of the Efficacy of P16+LBP, HPV+LBP, and P16+HPV

The combined screening methods of P16+LBP, HPV+LBP, and P16+HPV had sensitivities of 98.45%, 96.90%, and 99.22%, respectively. Their specificities were 80.29%, 63.42%, and 64.33%, respectively (Table 4).

The ability of P16+LBP and HPV+LBP screening methods to detect HSIL+ lesions was compared using the χ^2 test, and the specificity and positive predictive value were significantly different (χ^2 = 53.3623 and 14.9856, P < 0.0001, P < 0.05). In terms of the sensitivity and negative predictive value, the P values of the three were 0.1706 and 0.4847, all of which were > 0.05 (not statistically significant).

Table 3 Comparison of Screening Efficacy of Individual Tests

Screening Method		Clinical Diagnosis		Sensitivity	Specificity	Positive Predictive value	Negative Predictive value
		$HSIL^+$ (N)	$HSIL^-$ (N)				
P16	Positive	125	87	96.90%	88.72%	58.96%	99.42%
	Negative	4	684				
LBP	Positive	110	108	85.27%	85.99%	50.46%	97.21%
	Negative	19	663				
HR-HPV	Positive	117	239	90.70%	69.00%	32.87%	97.79%
	Negative	12	532				

Notes: Positive cases in clinical diagnosis refer to cases with histopathological results of $HSIL^+$ lesions (including HSIL, AIS, SCC, CADC, and other malignant lesions). Negative cases include not being biopsied (as no abnormality or suspicious findings were detected by colposcopy) or histopathologically diagnosed cases such as chronic cervicitis and LSI, polyps, or various benign proliferative causes lesions.

Abbreviations: LBP, liquid based cytology; HR-HPV, high-risk HPV infection; $HSIL^+$, high-grade cervical intraepithelial lesions and above (CIN2, CIN3, and carcinoma); $HSIL^-$, high-grade cervical intraepithelial lesions below (CIN1, normal).

Similarly, the χ^2 test was used to compare the ability of HPV+P16 and HPV+LBP to detect $HSIL^+$ lesions. The sensitivity, specificity, positive predictive value, and negative predictive value were compared, and the P values of the three were 0.8158, 0.1012, 0.0607, and 0.8204, all of which were > 0.05 (not statistically significant) (Tables 4–6).

Table 4 Comparison of the Ability of Combined Screening Methods in the Detection of $HSIL^+$ Lesions

Screening Method		Histopathological Result		Sensitivity (%)	Specificity (%)	Positive Predictive value (%)	Negative Predictive value (%)
		$HSIL^+$ Lesion	$HSIL^-$ Lesion				
P16+LBP	Positive	127	152	98.45	80.29	45.52	99.68
	Negative	2	619				
HPV+LBP	Positive	125	282	96.90	63.42	30.71	99.19
	Negative	4	489				
P16+HPV	Positive	128	275	99.22	64.33	31.76	99.80
	Negative	1	496				

Abbreviations: LBP, Liquid Based Cytology; HR-HPV, High-risk HPV infection; $HSIL^+$, High-grade cervical intraepithelial lesions and above (CIN2, CIN3, and carcinoma); $HSIL^-$, High-grade cervical intraepithelial lesions below (CIN1, normal).

Table 5 Comparison of the Ability of P16+LBP and HPV+LBP in the Detection of $HSIL^+$ Lesions

Screening Method	Sensitivity		Specificity		Positive Predictive value		Negative Predictive value	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
P16+LBP HPV+LBP	0.1706	0.6795	53.3623	<0.0001	14.9856	<0.0001	0.4847	0.4863

Table 6 Comparison of the Ability of HPV+P16, HPV+LBP in the Detection of $HSIL^+$ Lesions Screening Method

	Sensitivity		Specificity		Positive Predictive value		Negative Predictive value	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
HPV+P16 HPV+LBP	0.8158	0.3664	0.1012	0.7504	0.0607	0.8054	0.8204	0.3651

Table 7 Concordance Test Between Liquid-Based Cytological Detection for P16 and Histological Detection for P16

Histological Detection for P16	Liquid-Based Cytological Detection for P16	All Cases		Histopathological Results			
				Positive		Negative	
		N	%	N	%	N	%
Positive	Positive	131	64.85	116	94.31	15	18.99
	Negative	8	3.96	2	1.63	6	7.59
Negative	Positive	41	20.30	5	4.07	36	45.57
	Negative	22	10.89	0	0.00	22	27.85
Total		202	100.00	123	100.00	79	100.00

Concordance Test Between Liquid-Based Cytological Detection for P16 and Histological Detection for P16

In this study, the specimens of 202 patients were subject to both liquid-based cytological detection for P16 and histological detection for P16. Among them, 172 patients (85.15%) were positive for liquid-based cytological detection for P16, 139 (68.81%) were histologically positive for P16, and 123 (60.89%) were histopathologically high-grade lesions.

The concordance rate between histological detection for P16 and liquid-based cytological detection for P16 was 75.74% [(131+22)/202]. Results were discordant in 49 patients, including 8 histologically P16-positive and P16-negative in liquid-based cytological detection and 41 histologically P16-negative and P16-positive in liquid-based cytological detection.

Among the 123 patients with positive histopathological results, 5 cases (4.06%) in histological P16 detection, 2 cases (1.63%) in liquid-based cytological detection or P16, and 0 cases in both methods were missed diagnosis (Table 7).

Discussion

P16

P16 is a tumor suppressor gene closely related to cervical cancer occurrence, development, and prognosis.[12] The P16 protein can directly participate in the cell cycle regulation and suppress tumors by inhibiting cell proliferation and division. The expression of P16 in the normal cell cycle has an antiproliferative effect, and therefore, the normal level of P16 protein is unable to be detected by immunohistochemical means in terminally differentiated epithelial cells. However, in HPV-related precancerous lesions and carcinogenesis, the E7 oncoprotein, an essential factor for establishing and maintaining a malignant phenotype, is integrated into the host genome, complex to Rb proteins to inactivate the protein. As a result, the cells continue to increase and aggregate to form tumors, and correspondingly, the enhanced expression of P16 can be detected. Therefore, P16 is considered a surrogate marker for HPV infection and transformation and can be used to detect diseased cells before an abnormal cell morphology begins to take form. The application of P16 may help realize an early diagnosis.

P16 protein detection has been widely used in cervical biopsy and histopathology, especially in cases with difficult or puzzling interpretations based on the morphological diagnosis. P16 is often used as the standard for differential diagnosis. P16-negative samples are classified as LSIL, while P16-positive samples are classified as HSIL.[13] A high expression of P16 protein indicates viral integration activity, which is reflective of the ability of cells to become malignant and increase the aggressiveness of malignant tumors.[14,15] A test result of P16 positivity could indicate an increased risk for disease progression, thereby guiding the prognosis of high-grade lesions. This study found that among 900 women with complete and valid screening results, 61 (8.94%) were normal for cytological screening and p16-positive, 17 (50%) were LSIL-positive in cytological screening, and P16-positive and 17 (50%) were HSIL-positive for cytological screening and P16-positive. In 68 cases (98.55%), it can be seen that the positive rate of P16 tends to

increase with the increase of diagnostic grade in cytology (Table 1). Among those with normal cytology and pathology, the positive rate of P16 was 8.94%, while that of HSIL was 98.55%. With the aggravation of cervical lesions, the positive rate of P16 increased.

Efficacy of Individual Screening Methods of P16, LBP, HR-HPV

In this study, compared with HR-HPV, P16 had the highest sensitivity and negative predictive value in the detection of cervical HSIL$^+$ lesions (96.90% and 99.42%, respectively); these metrics were higher than those of HR-HPV (90.70% and 97.79%, respectively), and the specificity of P16 was higher than that of HR-HPV. The degree of HPV detection was 88.72%, much higher than the 69% of HR-HPV detection (Table 3); these findings were consistent with other reports.[16]

In this study, the specificity of P16 (88.72%) was slightly higher than that of LBP (85.99%) and much higher than that of HR-HPV (69.00%); these findings were in line with those reported by Song et al.[17] However, as the application of P16 in cervical cancer screening is not widely known, studies on reporting P16 as a screening target remain scarce, and the currently available research results are also inconsistent. Therefore, this warrants more in-depth, large-sample studies for verification.

This study found that the positive predictive value of HR-HPV for detecting cervical HSIL$^+$ lesions was only 32.77%, which was the lowest among the three screening methods. The negative predictive value of P16, LBP, and HR-HPV for high-grade lesions all exceeded 95%.

Almost all HPV-positive cervical cancers and high-grade lesions showed positive expression of P16. Similar to Ishikawa et al,[18] P16 detected by liquid-based cytology was positively correlated with cervical diseases and was an effective target for screening high-grade lesions. Considering the sensitivity and specificity, the efficiency of P16 screening in detecting high-grade lesions is better than that of HPV or LBP. As a screening index, it may help reduce the referral rate of colposcopy.

Combined Screening Method of HR-HPV and P16

With more in-depth research into the combined approaches to screening cervical cancer, there is an increase in the application of screening methods combining HPV with P16 screening in cervical cancer screening. Thomas also confirmed that P16 screening alone or in combination with HPV increased the detection rate of high-grade lesions[19] and could be used to triage HR-HPV-positive women in cervical cancer screening.[20]

The present study employed 900 study participants. As shown in Table 4, the P16+HPV screening method, which was used for screening cervical cancer and precancerous lesions, had the highest sensitivity (99.22%), with a specificity of 64.33%, which was lower than the specificity of the P16+LBP screening method (80.29%); the difference was statistically significant ($P < 0.005$). This study also implies that in screening cervical cancer and precancerous lesions, the application of combined screening methods can complement the advantages and disadvantages of the constituent methods and improve the efficacy of cervical cancer and precancerous lesions detection. Compared with the widely used HPV+LBP screening method, P16+HPV or P16+LBP have comparable or better application value in a clinical setting.

Comparison of the Efficacy of HPV+LBP, HPV+P16, and P16+LBP Screening Methods

This study compared the efficacy of three combined screening methods of P16+LBP, HPV+LBP, and P16+HPV in detecting HSIL+ lesions. The P16+HPV method had the highest sensitivity (99.22%), whereas the HPV+LBP method had the lowest sensitivity (96.90%). The P16+LBP method has the highest specificity (80.29%), and the HPV+P16 method has the lowest specificity (63.42%). Only these methods' specificity and positive predictive value were significantly different, but the sensitivity and negative predictive value were not. It can be seen that the combined screening method of HPV+P16 that did not involve the LBP method conducted by pathologists had the lowest specificity, but the LBP method had a high specificity.

As a primary screening method, cervical cytology is a non-invasive, fast, and highly specific method that plays an important role in early cervical cancer screening. However, problems such as poor result reproducibility, low sensitivity

[72.9% (70.7% to 75%)],[21] high rates of missed diagnosis and misdiagnosis[22] are common due to different material collection methods, insufficient quality control during the preparation and staining process, and differences in the subjectivity and experience of the examining doctors, which is common, especially in those with adenocarcinoma and a diagnosis of the squamous cell below the grade of HSIL.[23] However, P16 and TCT are taken from the same sample and examined by the same pathologist. The interpretation of P16 results is based on the color difference. Compared with TCT, the interpretation of cell morphology is simpler and more objective. It can realize automatic digital examination and improve reproducibility, reliability, and accuracy. Currently, there are not many options of reagents or products for immunochemical staining of exfoliated cervical cells for screening purposes. In addition, the staining procedure is practically manual, which results in frequent human errors. Limited options of staining techniques also result in increased interferences such as non-specific cross-staining and false positives, which may affect the interpretation of the results and impact the accuracy. The technologically innovative JY-6000 automatic detection system was employed in this study to make the staining background sharp and clear. By simply counting the stained cells for result interpretation, this instrument can make up for the inadequacies of cytological detection and deliver accurate and quick result interpretation, which is different from the LBP-based diagnosis that relies on the interpretation of cell morphology. Therefore, this instrument is suitable for areas with limited medical resources and professional cytopathologists.

Comparison of Screening Results of Histological Detection of P16 After Liquid-Based Cytological Detection for P16 on Colposcopy-Biopsy Specimens

The table of this study shows that the concordance rate of the screening results of histological P16 detection after liquid-based cytological detection for P16 on colposcopy-biopsy specimens was 75.74% [(131+22)/202 cases]. Although the results of 49 patients were inconsistent, among 123 patients with histopathologically confirmed high-grade lesions, 5 cases (4.06%) in histological P16 detection missed diagnosis, which was higher than 2 cases (1.63%) in liquid-based cytological detection for P16, indicating that the detection efficacy of liquid-based cytology for high-grade lesions was higher than that of the histopathological method. In addition, the invasive colposcopy-biopsy procedure is needed for acquiring specimens for histological P16 detection; therefore, it cannot be used as a screening method. On the other hand, liquid-based cytological detection for P16 adopts LBP to screen remaining specimens; therefore, this method is more economical, convenient, quick, and pain-free and can be used for screening early-stage cervical cancer.

The limitation of this study is that all patients were included in the three primary screenings, and patients who were directly referred for colposcopy after only one of the abnormal examinations were not included, so there was a certain selection bias. The sample size is large, but stratified management has not been achieved. The sample size can be increased in future studies, and the screening efficiency of risk stratification can be observed in large sample studies.

Conclusion

In conclusion, evaluating an effective biological screening index depends on whether it can detect the maximum number of cases and indirectly reduce the number of colposcopy referrals, thereby reducing unnecessary interventions or treatments. So far, missed diagnosis is inevitable in a particular screening method or combined screening methods. The selection of any one of the screening methods or combined screening method is made after weighing the risks and benefits. Compared with individual or combined screening methods that do not involve P16 detection, such as HR-HPV, LBP, and LBP+HPV, the application of P16 detection either individually or in combination with other methods, ie, P16 +HPV or P16+LBP, has higher efficacy in terms of convenience in operation, the interpretation of results, and ability in identifying high-grade lesions. Thus, P16 detection in cervical cancer screening is worthy of promotion owing to its application value in clinical settings.

Disclosure

The authors report no conflicts of intertest in this work.

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