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Evaluation of p16/Ki-67 dual staining in the detection of cervical precancer and cancer in China

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ABSTRACT

Background This study aimed to evaluate the clinical performance of p16/Ki-67 dual staining in the detection of cervical intraepithelial neoplasia grade 2 or 3 or worse (CIN2+/CIN3+) in Chinese women.

Methods Cervical exfoliated cells were collected from 537 eligible women and were used for liquid-based cytology (LBC), p16/Ki-67 dual staining, and human papillomavirus (HPV) DNA testing. All women received colposcopy with biopsies taken at abnormal sites. Histopathological diagnoses were used as the gold standard.

Results p16/Ki-67 staining had a positivity rate of 43.58% overall; the rate increased significantly with histological severity ($p < 0.001$). The sensitivities of p16/ki-67 for detecting CIN2+ and CIN3+ were 88.10% and 91.30%, respectively. Compared with high-risk HPV (HR-HPV), sensitivity of p16/Ki-67 was lower for detecting CIN2+ (88.10% versus 95.71%), but similar for detecting CIN3+ (91.30% versus 96.27%). Specificities of p16/Ki-67 were 85.02% for detecting CIN2+ and 76.86% for detecting CIN3+, values similar to those for LBC (84.71% for CIN2+, 80.05% for CIN3+) but higher than those for HR-HPV (62.77% for CIN2+, 71.25% for CIN3+). All the tests performed better in women > 30 years. With respect to the performance of triage for women with ASC-US, sensitivities of p16/Ki-67 were 86.36% for detecting CIN2+ and 83.33% for detecting CIN3+, values similar to those of HR-HPV. However, specificities of p16/Ki-67 were both higher than those of HR-HPV (85.96% versus 67.54% for CIN2+, 79.84% versus 62.90% for CIN3+).

Conclusion P16/Ki-67 dual staining could probably provide an optional method for China's national cervical cancer screening, and could also be considered as an efficient method of triage for managing women with ASC-US.

1. Introduction

Cervical cancer is the fourth most common cancer affecting women worldwide, with an estimated number of 528,000 new cases and 266,000 deaths in 2012. Of these, about 85% occur in less developed regions, accounting for 12% of all cancers in females [1]. Fortunately, cervical cancer is highly preventable through comprehensive prevention strategies [2,3]. Between 2009 and 2016 the Chinese government has provided nationwide free cervical cancer screening for women in rural areas (covering 60 million women) using visual inspection with acetic acid/visual inspection with Lugol's iodine solution (VIA/VILI), or the Papanicolaou (Pap) test, or a human papillomavirus (HPV) test (only in some pilot sites) [4]. However, owing to its large population, there were still 98,900 new cases and 30,500 deaths from cervical cancer in China in 2015 [5].

Recently, the three prophylactic HPV vaccines Cervarix, Gardasil 4

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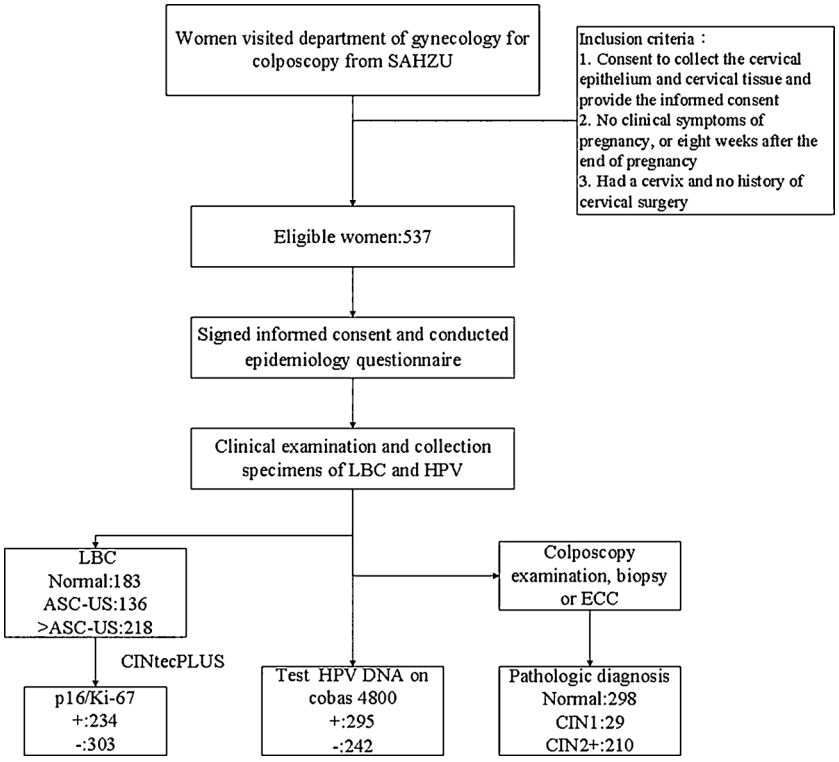

Fig. 1. The flowchart of study. LBC, liquid-based cytology; ECC, endocervical curettage; ASC-US, atypical squamous cells – undetermined significance; CIN1/2+: cervical intraepithelial neoplasia grade 1/2 or worse.

and Gardasil 9 have been successively approved by the China Food and Drug Administration [6–8]. Following this, the prevention of cervical cancer in mainland China will enter into a new era. However, at the present stage cervical cancer screening remains the main preventive measure in mainland China. It has been reported that there are large disparities in the disease burden of cervical cancer in different geographic areas [5]. Considering the unbalanced development of the regional economy, healthcare infrastructure and public health awareness, cervical cancer screening strategies vary in different areas. Visual-based methods are subjective, and their accuracy relies mainly on the experience of the performer [9]. Due to their intrinsic limitations—such as low reproducibility [10]—successful cytology-based screening programs require well-trained cytologists, repeated testing, high population coverage, and quality control procedures [11] which are difficult to achieve in areas low in resources. High false-positive rates of HPV-based screening have resulted in unnecessary colposcopy referrals and overtreatment, also increasing the mental burden for women [12]. It is therefore urgent to find proper biomarkers to improve the predictive performance of primary screening techniques and to improve the balance between sensitivity and specificity.

$P16^{INK4a}$ (p16) is a cyclin-dependent kinase inhibitor which has been proven to be significantly overexpressed in transforming infections with oncogenic types of HPV and is recognized to be a surrogate marker for cervical precancers [13]. At present, pathologists have used p16 immunohistochemistry staining as an adjunctive diagnostic method to improve the accuracy of cervical intraepithelial neoplasia (CIN) diagnosis [14]. However, p16 overexpression may also be observed in tubal metaplasia and atrophic cells as well as in normal columnar cells from the cervix, which leads to poor specificity [15]. Ki-67 is a nuclear antigen and a cellular proliferation biomarker expressed in all cell cycle phases except G0 [16]. The expression of p16 and Ki-67 is mutually exclusive in normal cells. However, the simultaneous expression of p16 and Ki-67 within a cell would be indicative of cervical epithelial cell transformation which may progress to cancer. Therefore, using p16/Ki-67 dual staining as a cervical cancer screening technique could be simple, objective, and highly efficient.

This study was conducted to assess the clinical performance of p16/Ki-67 as a marker for detecting cervical precancer and cancer in a hospital-based population of women, and aimed to provide evidence of the suitability of p16/Ki-67 dual staining as a screening strategy in China.

2. Materials and methods

2.1. Study population

This was a hospital-based study. A total of 537 eligible women aged 20–79 years who visited the gynecology department for colposcopy at the Second Affiliated Hospital of Zhengzhou University (SAHZU) were enrolled. Inclusion criteria were: (1) providing informed consent including consent to the collection of cervical epithelium and cervical tissue; (2) having no clinical symptoms of pregnancy, or there being 8 weeks since the end of pregnancy; and (3) having an intact cervix and no history of cervical surgery such as hysterectomy. Women who lacked any of the above criteria were excluded. The study was approved by the Institutional Review Board (IRB) of the Henan Cancer Hospital (HCH).

2.2. Study procedures

Gynecologists described the purpose and significance of the work to the women meeting the criteria. Women who agreed to participate in the project provided their informed consent. The gynecologists then conducted an epidemiological questionnaire with the women, carried out a gynecological examination, and collected specimens of cervical exfoliated cells by broom brush; the specimens were transferred to PreservCyt® liquid and stored at 4 °C. After that, gynecologists performed a colposcopy and biopsied tissues at abnormal sites; endocervical curettage (ECC) was carried out if colposcopy was not satisfactory. Fig. 1 shows the details of the study procedure.

Table 1
Human papillomavirus (HPV) and p16/ ki-67 detection rates in different pathological and cytological results.

	Total	HPV16/18 +		HPV–Other +		HR-HPV +		p16/Ki-67 +	
		n	%	n	%	n	%	n	%
Total	537	168	31.28	169	31.47	295	54.93	234	43.58
Cytology:									
Normal	183	13	7.10	34	18.58	42	22.95	26	14.21
ASC-US	136	21	15.44	41	30.15	57	41.91	35	25.74
ASC-H	22	18	81.82	6	27.27	21	95.45	17	77.27
AGC	7	2	28.57	3	42.86	4	57.14	6	85.71
LSIL	81	27	33.33	46	56.79	64	79.01	51	62.96
HSIL	75	58	77.33	25	33.33	74	98.67	68	90.67
SCC	31	27	87.10	14	45.16	31	100.00	29	93.55
AIS	2	2	100.00	0	0.00	2	100.00	2	100.00
Pathology:									
Normal	298	23	7.72	59	19.80	76	25.50	39	13.09
CIN1	29	8	27.59	10	34.48	18	62.07	10	34.48
CIN2	49	16	32.65	34	69.39	45	91.84	38	77.55
CIN3	111	80	72.07	50	45.05	106	95.50	99	89.19
SCC	43	35	81.40	14	32.56	43	100.00	42	97.67
ADC	7	6	85.71	1	14.29	6	85.71	6	85.71

ASC-US, atypical squamous cells – undetermined significance; ASC-H,atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; AGC, atypical glandular cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous-cell carcinoma; AIS, adenocarcinoma in situ; ADC, adenocarcinoma; LBC, liquid-based cytology; CIN, cervical intraepithelial neoplasia; CIN2 +, cervical intraepithelial neoplasia grade 2 or worse; CIN3 +, cervical intraepithelial neoplasia grade 3 or worse.

2.3. HPV DNA testing

Specimens of cervical exfoliated cells were transported to the Cancer Institute and Hospital, Chinese Academy of Medical Sciences (CICAMS), to carry out the cobas 4800 HPV testing (Roche, Basel, Swit), liquid-based cytology (LBC; ThinPrep; Hologic-Cytye, Marlborough, MA, USA), and p16/Ki-67 dual staining (CINtec PLUS; Roche mtm laboratories, Mannheim, Germany). Cobas HPV DNA testing can simultaneously detect 14 types of high-risk HPV (HR-HPV)—16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68—and report results of HPV 16/18 respectively based on the amplification of target HPV DNA by PCR. All procedures were performed according to the recommendations of the manufacturer.

2.4. p16/Ki-67 dual staining

P16/Ki-67 dual staining utilized an immunohistochemical method which can detect p16 and Ki-67 protein at the same time. P16/Ki-67 immunostains were reviewed by trained cytotechnologists in CICAMS. When the cervical exfoliated cells were transported to CICAMS, they were made into two slides. One was for LBC diagnosis and the other was for p16/Ki-67 dual staining. Samples with one or more cervical cells with red cytoplasm (p16) and yellow or brown nuclei (Ki-67) were called positive by a trained cytologist. If there were no double-stained cells the sample was called negative.

2.5. Cytological diagnoses

For LBC the Bethesda system for reporting cervical cytology was used by a senior cytotechnologist at CICAMS. LBC results were considered negative when the results were negative for an intraepithelial lesion or malignancy (NILM) and atypical squamous cells – undetermined significance (ASC-US). All other results were considered positive.

2.6. Histological diagnoses

Biopsy and ECC tissues were sent to SAHZU for histopathological diagnoses by the CIN reporting system. Then a histopathologist from HCH checked all the tissues. If no agreement was achieved, the tissue would be sent to CICAMS for adjudication. The final diagnosis for each woman was based on the worst result of the review panel. All the detection and diagnostic processes were blind.

2.7. Statistical analysis

Continuous and categorical variables were compared using the *t*-test and chi-square test, respectively. Both absolute estimates of positive or negative rates of HPV, p16/Ki-67 and LBC, sensitivity and specificity as well as positive predictive value (PPV) and negative predictive value (NPV) were calculated. Estimates were provided with their 95%CIs using the Wald test by OpenEPi Version 3 (www.OpenEpi.com), open public-source epidemiological statistics for public health. Differences between p16/Ki-67 detections among different HPV types were calculated by chi-square test or Fisher's exact test. Statistical significance was assessed by two-tailed tests with an alpha level of 0.05. SPSS 19.0 was used for all the analyses.

3. Results

In total 537 eligible women were enrolled. The mean age of the women was 43.88 ± 10.97 years (range 20–79 y). Among them, 218 women (40.60%) had positive LBC results (> ASC-US). Based on histopathological diagnoses, there was a total of 209 subjects (38.92%) with CIN2 (48 women, 8.94%), CIN3 (111 women, 20.67%), squamous carcinoma of cervix (SCC) (43 women, 8.00%), or cervical adenocarcinoma (ADC) (seven women, 1.30%).

As shown in Table 1, the overall positivity rate for p16/Ki-67dual staining (43.58%) was lower than that for HR-HPV infection (54.93%) ($\chi^2 = 188.30$, $p < 0.001$) and higher than that for LBC > ASC-US (40.60%) ($\chi^2 = 191.10$, $p < 0.001$). Positivity rates for p16/Ki-67 dual staining increased significantly with histological severity ($\chi^2 = 288.84$, $p < 0.001$). In SCC and ADC, it was up to 97.67% and 85.71%, respectively. With regard to HR-HPV infection, the positivity rate of HR-HPV was highest in SCC (100.00%). For HPV16/18 infection, it was highest in ADC (85.71%). For the other 12 types of HR-HPV infection it was highest in women with CIN2 (69.39%).

Table 2 shows the positivity rate of p16/Ki-67 dual staining in different statuses of HR-HPV infection. P16/Ki-67 expressed in HR-HPV infected women (70.17%) was significantly higher than in HR-HPV

Table 2
Positive rates of p16/Ki-67 dual staining in different human papillomavirus (HPV) infection statuses.

HPV infection		p16/Ki-67				χ^2	p
		+		–			
		n	%	n	%		
HPV16/18	+	135	80.36	33	19.64	134.53	< 0.001
	–	99	26.83	270	73.17		
HPV-other	+	104	61.54	65	38.46	32.36	0.001
	–	130	35.33	238	64.67		
HR-HPV	+	207	70.17	88	29.83	188.30	< 0.001
	–	27	11.16	215	88.84		

negatives (11.16%) (p < 0.001). Stratified by types, the p16/Ki-67 positivity rate (80.36%) in HPV16/18 infections (168) was nearly three times that of the HPV16/18 negatives (369) (26.83%, $p < 0.001$), and the p16/Ki-67 positivity rate (61.54%) in HPV-other infected women (169) was close to twice that of the HPV-other negatives (368) (35.33%, $p < 0.001$).

Sensitivities, specificities, PPVs, and NPVs for all screening methods for detecting CIN2+ or CIN3+ are shown in Tables 3–5. In all age groups, sensitivities of p16/ki-67 dual staining in detecting CIN2+ and CIN3+ were 88.10% and 91.30%, respectively. Compared to HR-HPV, sensitivity was lower when detecting CIN2+ (88.10% versus 95.71%, $p < 0.05$) and had a similar performance when CIN3+ was the endpoint (91.30% versus 96.27%, $p > 0.05$). Compared to LBC, sensitivities of p16/Ki-67 were similar (88.10% versus 80.00% for CIN2+, 91.30% versus 88.82% for CIN3+, all $p > 0.05$). Specificities of p16/ki-67 for the detection of CIN2+ and CIN3+ were similar to those of LBC (85.02% versus 84.71% for CIN2+, 76.86% versus 80.05% for CIN3+, all $p > 0.05$) but higher than those of HR-HPV (85.02% versus 71.25%, 76.86% versus 62.77%, all $p < 0.05$).

When stratified by age, sensitivity and specificity of p16/Ki-67, HR-HPV and LBC in detecting CIN2+ and CIN3+ were similar (all $p > 0.05$) in women aged < 30 years. In women aged ≥30 years, sensitivity of p16/ki-67 in detecting CIN2+ and CIN3+ paralleled that of HR-HPV and LBC (all $p > 0.05$). Specificities of p16/Ki-67 dual staining in detecting CIN2+ and CIN3+ were higher than those of HPV (85.97% versus 72.66% for CIN2+, 77.02% versus 63.35% for CIN3+, all $p < 0.05$) but similar to those of LBC (85.97% versus 85.97% for CIN2+, 77.02% versus 81.06% for CIN3+, all $p > 0.05$).

One hundred and thirty-six women were diagnosed with ASC-US, and 22 women in this group were finally diagnosed with CIN2+. Sensitivity of p16/ki-67 was 86.36% for detecting CIN2+ and 83.33% for detecting CIN3+, similar to that of HR-HPV (95.45%, 100.00%, $p > 0.05$). However, in both cases specificities were higher than for HR-HPV (85.96% versus 67.54% for CIN2+, 79.84% versus 62.90% for CIN3+). PPVs and NPVs of HR-HPV and p16/ki-67 in detecting CIN2+ and CIN3+ were similar (Table 5).

4. Discussion

Recently, primary HPV DNA testing—alone or conjunction with cytology–has been used in cervical screening in many countries [17–19]. HR-HPV DNA testing has a high sensitivity and can find more than 90% of CIN2+ cases according to the results of our study and those of previous studies [20,21]. However, because most HPV infection is transient, and only persistent infection can lead to cancer, the specificity of HR-HPV in detecting CIN2+ (71.25%) is lower than that of LBC (84.71%, $p < 0.05$), as shown by our study and a previous one [22]. Low specificity will directly increase the burden of referral to colposcopy and the psychological burden for the women. With regard to cytology in China, different areas have different diagnostic levels. A study by Pan et al. showed that the accuracy of CIN2+ diagnosis—by cytology and histology—was 76.74% [23], while in the rural area of Wuhan it was only 22.15% [24]. This means that cancer or precancer will be missed when cytology is the primary cervical cancer screening method. HPV-based and cytology-based screening strategies have their own shortcomings. To settle these problems, p16/Ki-67 dual staining has been of interest since its development, as staining is detected only in abnormal cells and can easily be observed under the microscope even by non-professional cytologists.

Theoretically, p16/Ki-67 co-expression is related to HPV infection. HPV virus—especially HPV 16/18—interferes with the cell regulation mechanism through integration of its DNA into the DNA of the host cell [25,26]. The abnormal cell then expresses p16 and Ki-67 simultaneously, transforming the activity of the E7 oncoprotein through inactivation of the tumor-suppressor function of the retinoblastoma protein [27,28]. The results of our study showed that the p16/Ki-67 positivity rate in women infected with HR-HPV was six-fold higher than in HPV negatives, and three-fold higher in women infected with HPV16/18 than in negatives. This is consistent with the findings of other studies [29,30], and indicates a strong relationship between p16/Ki-67 co-expression and HR-HPV infection, especially for women infected with HPV16/18. In our study we also observed that positivity rates of p16/Ki-67 increased with the grade of histological severity; this is in agreement with the findings of previous studies [31,32].

Many studies have recommended HR-HPV testing as the primary cervical screening method for developing countries [17,33]. Our study shows that p16/Ki-67 dual staining can detect most cervical precancers

Table 3
Clinical performance characteristics of p16/Ki-67 dual staining, high-risk human papillomavirus (HR-HPV), and liquid-based cytology (LBC) for detection of CIN2+ in women.

	Sensitivity		Specificity (%)	PPV (%)	NPV (%)
	n/N	(%)			
Women all ages (n = 537):					
HPV	201/210	95.71 (92.06, 97.73)	71.25 (66.12, 75.89)	68.14 (62.61, 73.19)	96.28 (93.08, 98.03)
LBC	168/210	80.00 (74.07, 84.85)	84.71 (80.41, 88.21)	77.06 (71.04, 82.15)	86.83 (82.68, 90.11)
p16/Ki-67	185/210	88.10 (83.02, 91.81)	85.02 (80.74, 88.48)	79.06 (73.40, 83.78)	91.75 (88.10, 94.35)
Women aged < 30 (n = 66):					
HPV	15/17	88.24 (65.66, 96.71)	63.27 (49.27, 75.34)	45.45 (29.84, 62.01)	93.94 (80.39, 98.32)
LBC	13/17	76.47 (52.74, 90.45)	77.55 (64.12, 86.98)	54.17 (35.07, 72.11)	90.48 (77.93, 96.23)
p16/Ki-67	14/17	82.35 (58.97, 93.81)	79.59 (66.36, 88.52)	58.33 (38.83, 75.53)	92.86 (80.99, 97.54)
Women aged ≥30 (n = 471):					
HPV	186/193	96.37 (92.70, 98.23)	72.66 (67.14, 77.57)	70.99 (65.23, 76.15)	96.65 (93.25, 98.37)
LBC	155/193	80.31 (74.13, 85.31)	85.97 (81.40, 89.56)	79.90 (73.70, 84.93)	86.28 (81.73, 89.84)
p16/Ki-67	171/193	88.60 (83.34, 92.35)	85.97 (81.40, 89.56)	81.43 (75.62, 86.11)	91.57 (87.57, 94.37)

PPV, positive predictive value; NPV, negative predictive value.

Table 4
Clinical performance characteristics of p16/Ki-67 dual staining, high-risk human papillomavirus (HR-HPV), and liquid-based cytology (LBC) for detection of CIN3+ in women.

	Sensitivity		Specificity (%)	PPV (%)	NPV (%)
	n/N	(%)			
Women all ages (n = 537):					
HPV	155/161	96.27 (92.11, 98.28)	62.77 (57.77, 67.50)	52.54 (46.85, 58.17)	97.52 (94.70, 98.86)
LBC	143/161	88.82 (83.02, 92.81)	80.05 (75.72, 83.78)	65.60 (59.07, 71.58)	94.36 (91.26, 96.40)
p16/Ki-67	147/161	91.30 (85.93, 94.75)	76.86 (72.34, 80.84)	62.82 (56.47, 68.76)	95.38 (92.39, 97.23)
Women aged < 30 (n = 66):					
HPV	11/12	91.67 (64.61, 98.51)	59.26 (45.97, 71.32)	33.33 (19.75,50.39)	96.97 (84.86,99.46)
LBC	10/12	83.33 (55.20, 95.30)	74.07 (61.07, 83.88)	41.67 (24.47, 61.17)	95.24 (84.21, 98.68)
p16/Ki-67	11/12	91.67 (64.61, 98.51)	75.93 (63.05, 85.36)	45.83 (27.89, 64.93)	97.62 (87.68, 99.58)
Women aged ≥30 (n = 471):					
HPV	144/149	96.64 (92.39, 98.56)	63.35 (57.96, 68.43)	54.96 (48.91, 60.87)	97.61 (94.52, 98.97)
LBC	133/149	89.26 (83.27, 93.28)	81.06 (76.42, 84.96)	68.56 (61.72, 74.68)	94.22 (90.82, 96.41)
p16/Ki-67	136/149	91.28 (85.65, 94.83)	77.02 (72.12, 81.28)	64.76 (58.09, 70.91)	95.02 (91.67, 97.07)

PPV, positive predictive value; NPV, negative predictive value.

(88.10% for CIN2+, 91.30% for CIN3+) similarly to HR-HPV (95.71% for CIN2+, 88.10% for CIN3+), and also demonstrates specificities (85.02% for CIN2+ and 76.86% CIN3+) similar to those of LBC (84.71% for CIN2+, 80.05% for CIN3+). This is in accordance with previous studies [31,34]. However, the previous studies differed from our study in that they showed no significant difference between sensitivities and specificities of p16/Ki-67 and HR-HPV as well as LBC. This may be due to a different study design and a different population. Higher specificities can decrease the burden of colposcopy. If p16/Ki-67 dual staining were used as the triage strategy, colposcopy referral rates would decrease by 11.36%; 16 women with CIN2+ would be missed compared to all HR-HPV after triage, but the detection rate for CIN2+ would increase by 10.92%.

HPV is common in young women, but most infections are temporary and will be cleared [35]. Therefore, practice bulletin No.157 regarding the prevention and early detection of cervical cancer recommends that women < 30 years old need only cytology [36]. Results of this study were stratified by age group and showed that sensitivities and specificities of p16/Ki-67 dual staining in detecting CIN2+ or CIN3+ were similar to those of LBC in women aged < 30 years. It suggests that young women can receive p16/Ki-67 dual staining to avoid the subjectivity of diagnosis by cytology. Hence, under the current circumstances, p16/Ki-67 dual staining is a promising alternative method for cervical screening. A systematic literature review also showed that p16/Ki-67 dual staining is more sensitive but slightly less specific than cytology [32].

Performance of p16/Ki-67 for triage in women diagnosed with ASC-US was also analyzed in this study. Women diagnosed with ASC-US make up a large burden for the colposcopy doctor. Recently, HPV DNA testing as a management measure was used to triage these women attending for colposcopy [37]. However, it can still lead to over-referral because of its low specificity. In these women, sensitivities of p16/Ki-67 in detecting CIN2+ and CIN3+ were similar to those of HR-HPV, and specificities were 85.96% (for CIN2+) and 79.84% (for CIN3+) respectively, higher than those for HR-HPV ($p < 0.05$). This supports the idea that p16/Ki-67 dual staining could be applied to triage women with undefined and abnormal LBC results, which is consistent with the findings of previous studies [38–40].

With regard to weaknesses of the study, it is important to note that the results cannot be fully generalized to cervical screening in the whole population because women of this study were hospital-based. The fraction of women aged < 30 years was small; therefore, the representativeness of the results is limited and it's hard to avoid some chance factors. Furthermore, this was a cross-sectional study and cannot reflect the long-term effects of p16/Ki-67 dual staining.

In summary, p16/Ki-67 dual staining shows a good clinical performance for detecting cervical precancer and cancer, with a sensitivity similar to that of HR-HPV and a specificity similar to that of LBC. It could also be a promising method for managing women diagnosed with ASC-US. Because of its advantages over HR-HPV and LBC—such as low price and simple operation—p16/Ki-67 dual staining could be an alternative method for cervical cancer screening in China, especially in rural areas.

5. Conclusion

P16/Ki-67 dual staining probably provides an optional method for China's national cervical cancer screening and could also be considered as an efficient triage method for managing women with ASC-US.

6. Authorship contribution

Shao-Kai Zhang designed the study, performed the statistical analysis and amended the manuscript. Man-Man Jia drafted the manuscript

Table 5
Clinical performance characteristics of p16/Ki-67 dual staining, high-risk human papillomavirus (HR-HPV) and liquid-based cytology (LBC) for detection of CIN2+ or CIN3+ in women with atypical squamous cells – undetermined significance (ASC-US).

	Sensitivity		Specificity (%)	PPV (%)	NPV (%)
	n/N	(%)			
CIN2+:					
HPV	21/22	95.45 (78.20, 99.19)	67.54 (58.50, 75.44)	36.21 (25.05, 49.07)	98.72 (93.09, 99.77)
p16/Ki-67	19/22	86.36 (66.66, 95.25)	85.96 (78.41, 91.17)	54.29 (38.19, 69.53)	97.03 (91.63, 98.98)
CIN3+:					
HPV	12/12	100.00 (75.75, 100.00)	62.90 (54.13, 70.90)	20.69 (12.25, 33.77)	100.00 (95.31, 100.00)
p16/Ki-67	10/12	83.33 (55.20, 95.30)	79.84 (71.93, 85.95)	28.57 (16.33, 45.06)	98.02 (93.07, 99.46)

PPV, positive predictive value; NPV, negative predictive value.

and checked the data and statistical analysis. Dong-Mei Zhao, Ze-Ni Wu and Guo Zhen did the experiment and amended the manuscript. Yu-Ling Liu, Pei-Pei Guo carried out the data collection. Qiong Chen, Xiao-Qin Cao and Shu-Zheng Liu checked the data and amended the manuscript. Wen Chen and Xi-Bin Sun participated in the conception and design of the study, and helped with coordination. All authors read and approved the final manuscript.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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