Evaluation of p16/Ki-67 Dual-Stained Cytology in Triaging HPV-Positive Women during Cervical Cancer Screening

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ABSTRACT

Background: We aimed to evaluate the utility of p16/Ki-67 dual-stained cytology for triaging human papillomavirus (HPV)-positive women.

Methods: HPV-positive women ages ≥ 21 years were recruited in a multicenter prospective observational study between May 2016 and May 2017. The clinical performance of dual-stained cytology, with or without HPV16/18 genotyping, was evaluated for all HPV-positive women to detect cervical intraepithelial neoplasia grade 2 or worse (CIN2+).

Results: 846 HPV-positive women ages ≥ 21 years with valid cervical biopsies were enrolled for this study. For CIN2+ detection, dual-stained cytology showed statistically higher specificity (85.28%) than Pap cytology (80.00%, $P < 0.001$) and HPV16/18 genotyping (72.36%, $P < 0.001$), while the sensitivity of dual-stained cytology (63.49%) remained comparable with that of Pap cytology (61.90%, $P = 0.832$) and HPV16/18 genotyping (61.90%, $P = 0.897$). HPV16/18 genotyping in combination with dual-stained cytology was more specific (62.50% vs. 58.06%, $P < 0.001$), while it showed similar sensitivity (86.51% vs. 85.71%, $P = 1.000$), as compared with HPV16/18 genotyping in combination with Pap cytology. Similar patterns were also observed for CIN3+.

Conclusions: p16/Ki-67 dual-stained cytology, either alone or in combination with HPV16/18 genotyping, showed a good stratification with high specificity and comparable sensitivity for HPV-positive women.

Impact: This is one of the few studies that has evaluated the performance of dual-stained cytology for triaging HPV-positive women in China. The higher specificity and comparable sensitivity of dual-stained cytology in comparison with Pap cytology in the detection of CIN2+ or CIN3+ is of vital importance to developing countries, where Pap cytology faces many challenges.

Introduction

A persistent cervical infection with high-risk papillomavirus is an indispensable cause of cervical cancer (1). The high sensitivity of human papillomavirus (HPV) testing and its reassurance of a low risk of cervical cancer in HPV-negative women has fostered a shift from cytology-based screening toward HPV testing as the primary cervical cancer screening strategy (2, 3). However, many HPV infections are transient and can be eliminated by the body's immune system. As such, it is neither feasible nor efficient for all HPV-positive women to have a referral for colposcopy. Therefore, additional triage strategies are needed to distinguish those HPV-positive women who are at a high risk and need colposcopy from those who can safely return to routine screening. According to the current triage strategies for primary cervical screening, women with HPV16/18–positive results are referred to colposcopy and women positive for 12 other HPV genotyping are further triaged by Pap cytology (2). However, the reliance on morphologic assessment and relative subjectivity, for which high expertise is required, limits the overall effectiveness of Pap cytology as the optimal "second line" triage test. Thus, there is a need to investigate new triage strategies for primary HPV screening.

$P16^{INK4a}$ (p16)/Ki-67 dual-stained cytology has shown promise as a triage for HPV-positive women (4). The simultaneous detection of the overexpression of p16, a cell-cycle arrest protein under normal physiologic conditions, and Ki-67, the cell proliferation marker, within the same cervical epithelial cell indicates HPV-induced deregulation of cell cycle (5). A good to excellent reproducibility of this morphology-independent biomarker has been observed with almost identical clinical performance of novice evaluations as compared with reference evaluations (6). The p16/Ki-67 dual-stained cytology combines superior specificity and high sensitivity for detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+; refs. 7, 8), which is now used as a triage strategy for managing the HPV-positive women during primary cervical screening (9–11), for the women with abnormal Pap cytology (12), and for the HPV-positive women with normal Pap cytology (13). However, few studies have compared concurrently the performance of

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p16/Ki-67 dual-stained cytology, Pap cytology, and HPV16/18 genotyping in Chinese women during primary cervical screening.

Our study aims to evaluate the clinical performance of p16/Ki-67 dual-stained cytology, Pap cytology, HPV16/18 genotyping, and the combination thereof, for the detection of CIN2+ or CIN3+ in a population of HPV-positive Chinese women.

Materials and Methods

Study population and procedures

This prospective observational study was nested into a routine cervical cancer screening program from May 2016 to May 2017. Females were eligible if ages ≥ 21 years and undergoing routine cervical screening at the Outpatient Department of Obstetrics and Gynecology from nine hospitals, including Renji Hospital of Shanghai Jiao Tong University (Shanghai, China), Shanghai Pudong New area People's Hospital (Shanghai, China), Shanghai Pudong Hospital (Shanghai, China), Shanghai Gongli Hospital (Shanghai, China), Shanghai Punan Hospital (Shanghai, China), Shanghai Pudong New area Maternal and Child Health Hospital (Shanghai, China), Shanghai ZhouPu Hospital (Shanghai, China), Dongfang Hospital of Tongji University (Shanghai, China), and Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine (Shanghai, China). Women with negative HPV results were advised to return to regular screening after 3 years and the positive ones were required to take pap cytology and dual-stained cytology in 2–4 weeks afterward. Women with positive HPV and valid pap cytology and dual-stained cytology results were then referred to colposcopy, which entailed cervical biopsy 2–4 weeks after the cytology tests had taken place. The cervical histologic results were obtained 1–2 weeks after colposcopy. Females were excluded from the study in case of: (i) negative HPV results; (ii) no valid Pap cytology results; (iii) no valid dual-stained cytology results; (iv) no cervical biopsy histopathologic diagnosis; (v) having uterine or cervical resection; (vi) pregnancy. The study was approved by the institutional review board of Renji Hospital, School of Medicine, Shanghai Jiao Tong University (Shanghai, China). The written informed consent was obtained from all the participants.

HPV testing

A liquid-based cervical specimen with endocervical brush was collected from all participating women by the clinicians. The specimen was then used for HPV testing performed using Roche Cobas 4800 system, which included HPV 16, 18, and 12 other high-risk HPV subtypes. Women with negative HPV test results were excluded from our study and returned to routine screening, while a second liquid-based cervical sample was collected from the HPV-positive women by the clinicians for Pap cytology and p16/Ki-67 dual-stained cytology.

Pap cytology

Half of the second liquid-based cervical samples were processed and reported in the laboratory of the Department of Pathology using Thinprep method. The results were analyzed by Bethesda system (TBS) criteria as negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (HSIL; ASC-H) and HSIL (14). ASC-US and the above grade were defined as Pap cytology-positive.

p16/Ki-67 dual-stained cytology

Residual slides were prepared from the second cervical samples for p16/Ki-67 dual-stained cytology analysis using the CINtec PLUS Cytology Kit (Roche) by BenchMark ULTRA (Roche) in the laboratory of the Department of Pathology, according to the manufacturer's instructions. The slides were evaluated by an expert cytotechnologist who was blinded to the HPV and Pap cytology results. The cytotechnologist acquired both physician's medical license on pathology and certificate on cytology and received training on how to evaluate and interpret dual-stained slides by Roche. Samples with one or more cervical epithelial cells that simultaneously showed red nuclear immunostaining (Ki-67) and brown cytoplasmic immunostaining (p16) were classified as positive regardless of the morphologic appearance of the cells. Slides that did not meet the minimum squamous cellularity criteria (5,000 squamous cells for liquid-based preparations) were excluded from the analysis, as specified in the Bethesda system for

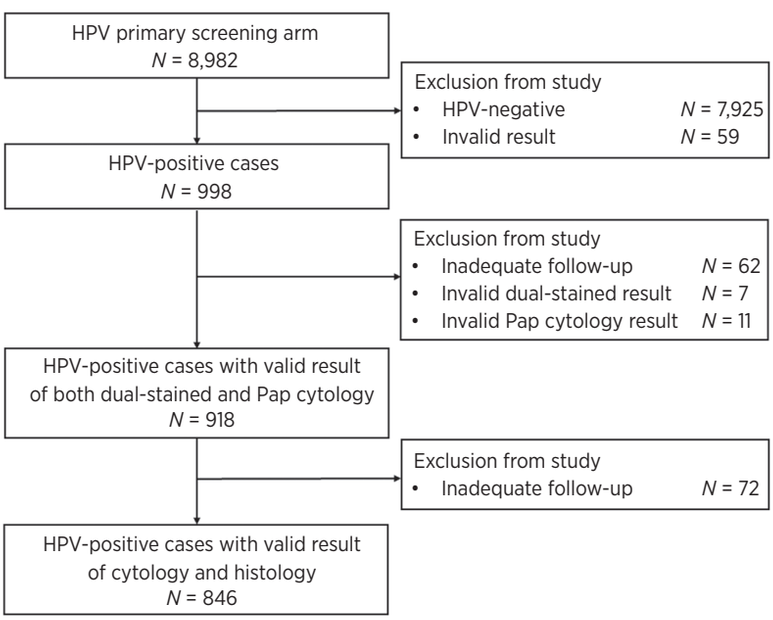

Figure 1.
Trial profile. Women with negative HPV results were advised to return to the regular screening, and the ones with positive results were required to take the dual-stained cytology and Pap cytology afterward. The HPV-positive women with valid dual-stained cytology and Pap cytology results were then referred to colposcopy and obtained the cervical histologic results.

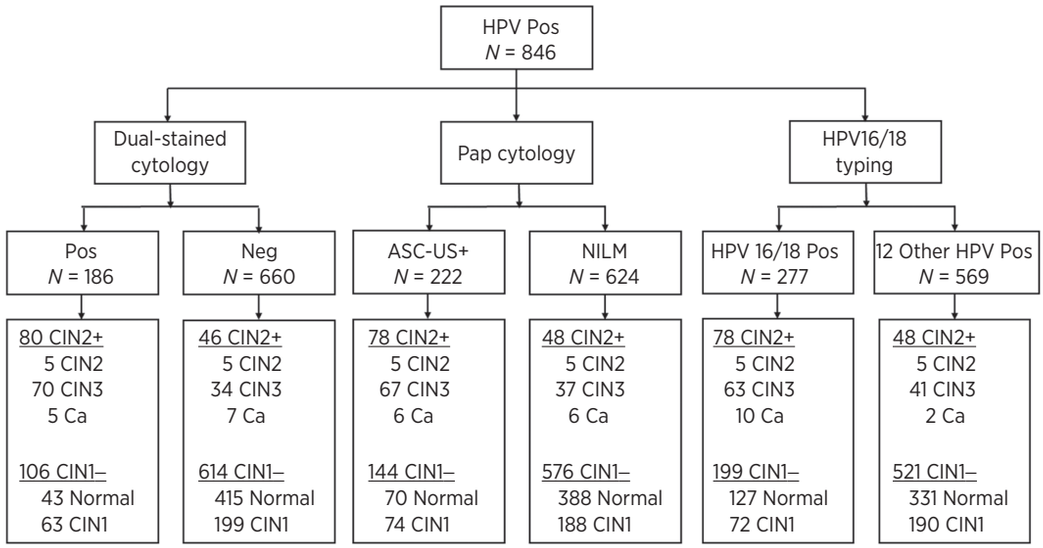

Figure 2.
Diagram of the study population. Pos, positive; Neg, negative; CIN 1–, CIN of grade 1 or negative for dysplasia. ASC-US+, ASC-US, and the above grade of Pap cytology.

reporting cervical cytology. Slides with excessive background staining were also considered invalid and excluded from the analysis.

Disease endpoint

All women that underwent colposcopy had at least one biopsy taken, with the majority of women receiving the multipoint biopsies to improve ascertainment of cervical precancer. Histologic results were evaluated on the basis of the CIN classification. We evaluated the endpoint as the detection of CIN2+ and CIN3+ separately.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of triage strategies were calculated using MedCalc Software (version 16.0). The sensitivity and specificity between groups were compared using an exact McNemar χ^2 test. The differences in PPV and NPV were evaluated by a marginal regression model using IBM SPSS software (version 21.0). Statistical significance was defined as $P < 0.05$. All statistical tests performed were two-sided.

Results

Baseline characteristics

A total of 846 HPV-positive women were enrolled in our study and the trial profile is shown in **Fig. 1**. The mean age of the 846 HPV-positive women was 41.82 ± 11.24 years. There was a total of 126 cases of pathologically confirmed CIN2+, including 10 cases of CIN2, 104

Table 1. P16/Ki-67 dual-stained cytology positivity by Pap cytology and histology results.

Cytology result	Total ($n = 846$) *n* (%)	Normal ($n = 458$) *n* (%)	CIN1 ($n = 262$) *n* (%)	CIN2 ($n = 10$) *n* (%)	CIN3 ($n = 104$) *n* (%)	Cancer ($n = 12$) *n* (%)
NILM	624 (73.76)	388 (84.72)	188 (71.76)	5 (50.00)	37 (35.58)	6 (50.00)
p16/Ki67+	47 (7.53)	15 (3.87)	20 (10.64)	1 (20.00)	11 (29.73)	0 (0.00)
HPV16/18+	188 (30.13)	108 (27.84)	50 (26.60)	3 (60.00)	22 (59.46)	5 (83.33)
ASC-US	85 (10.05)	43 (9.39)	27 (10.31)	2 (20.00)	12 (11.54)	1 (8.33)
p16/Ki67+	39 (45.88)	13 (30.23)	14 (51.85)	2 (100.00)	9 (75.00)	1 (100.00)
HPV16/18+	31 (36.47)	12 (27.91)	9 (33.33)	1 (50.00)	8 (66.67)	1 (100.00)
LSIL	68 (8.04)	17 (3.71)	39 (14.89)	1 (10.00)	10 (9.62)	1 (8.33)
p16/Ki67+	37 (54.41)	7 (41.18)	22 (56.41)	0 (0.00)	7 (70.00)	1 (100.00)
HPV16/18+	19 (27.94)	4 (23.53)	9 (23.08)	1 (100.00)	4 (40.00)	1 (100.00)
ASC-H	12 (1.42)	2 (0.44)	1 (0.38)	0 (0.00)	6 (5.77)	3 (25.00)
p16/Ki67+	8 (66.67)	1 (50.00)	0 (0.00)	0 (0.00)	5 (83.33)	2 (66.67)
HPV16/18+	4 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	2 (33.33)	2 (66.67)
HSIL	57 (6.74)	8 (1.75)	7 (2.67)	2 (20.00)	39 (37.50)	1 (8.33)
p16/Ki67+	55 (96.49)	7 (87.50)	7 (100.00)	2 (100.00)	38 (97.44)	1 (100.00)
HPV16/18+	35 (61.40)	3 (37.50)	4 (57.14)	0 (0.00)	27 (69.23)	1 (100.00)
Total	846 (100.00)	458 (54.14)	262 (30.97)	10 (1.18)	104 (12.29)	12 (1.42)
p16/Ki67+	186 (21.99)	43 (9.39)	63 (24.05)	5 (50.00)	70 (67.31)	5 (41.67)
HPV16/18+	277 (32.74)	127 (27.73)	72 (27.48)	5 (50.00)	63 (60.58)	10 (83.33)

cases of CIN3, and 12 cases of cervical carcinoma. There were 262 cases of CIN1 and 458 cases of normal pathology defined as CIN1− (**Fig. 2**). Among the 846 HPV-positive women, 202 were HPV16 positive, 75 were HPV18 positive, and 569 were 12 other HPV genotyping positive.

Dual-stain positivity by Pap cytology and histology results

Among all patients, 624 (73.76%) had NILM, 85 (10.05%) had ASC-US, 68 (8.04%) had LSIL, 12 (1.42%) had ASC-H, and 57 (6.74%) had HSIL, as shown in cytology results (**Table 1**). The dual stain positivity rate significantly increased with increasing severity of Pap cytology from 7.53% in HPV-positive women with NILM to 96.49% in women with HSIL. Similarly, the dual stain positivity rate increased from 9.39% in women with a normal histology, to 67.31% in women with CIN3 biopsy results. The dual stain positivity rate (186, 21.99%) was lower than the prevalence of abnormal Pap cytology results at an ASC-US threshold (222, 26.24%, $P = 0.041$; **Table 1**).

Diagnostic performance of dual-stained cytology for CIN2+ and CIN3+ detection

The sensitivity, specificity, PPV, and NPV of dual-stained cytology, Pap cytology, and HPV16/18 genotyping for detection of CIN2+ and CIN3+ are shown in **Table 2**. For detection of CIN2+, the specificity of dual-stained cytology was 85.28%, which was significantly higher than that of Pap cytology (80.00%, $P < 0.001$) and HPV16/18 genotyping (72.36%, $P < 0.001$). The sensitivity of dual-stained cytology, Pap cytology, and HPV16/18 genotyping were comparable: 63.49%, 61.90%, and 61.90% ($P > 0.05$), respectively. The PPV of dual-stained cytology was 43.01%, which was significantly higher than both Pap cytology (35.14%, $P = 0.001$) and HPV16/18 genotyping (28.16%, $P < 0.001$). The NPV of dual-stained cytology was 93.03%, which was significantly higher than that of Pap cytology (92.31%, $P < 0.001$) and HPV16/18 genotyping (91.56%, $P < 0.001$; **Table 2**). Similar patterns were observed for the CIN3+ endpoint, with higher specificity (84.79%), PPV (40.32%), and NPV (93.79%) for dual-stained cytology as compared with both Pap cytology and HPV16/18 genotyping (**Table 2**).

Diagnostic performance of combining HPV16/18 genotyping with dual-stained cytology

The utility of dual-stained cytology and Pap cytology as triage options for HPV-positive women was further assessed in combination with HPV16/18 genotyping. The combination triage strategies were implemented as follows: women positive for HPV16/18 were referred to colposcopy, while women with 12 other HPV genotyping positive results were further detected with either dual-stained cytology or Pap cytology. HPV16/18 genotyping combined with 12 other HPV genotyping with a further triage of dual-stained cytology exhibited a higher specificity (62.50% vs. 58.06% for CIN2+, $P < 0.001$; 61.92% vs. 57.53% for CIN3+, $P < 0.001$), PPV (28.76% vs. 26.34% for CIN2+, $P = 0.004$; 26.65% vs. 24.39% for CIN3+, $P = 0.006$), and NPV (96.36% vs. 95.87% for CIN2+, $P < 0.001$; 96.79% vs. 96.33% for CIN3+, $P < 0.001$) as compared with the combination with Pap cytology, while the sensitivity of both combination tests were identical (**Table 3**).

Number of colposcopies required for CIN2+ or CIN3+ detection using different triage strategies

Next, we compared the total number of colposcopies and the number of colposcopies detected per CIN2+ or CIN3+, as shown in **Table 4**. The dual-stained cytology triage strategy needed the lowest number of colposcopies detected per CIN2+ or CIN3+ ($n = 2.33$ for CIN2+, $n = 2.48$ for CIN3+). A combined triage option of

Table 2. Clinical performance of dual-stained cytology, Pap cytology, and HPV16/18 typing for detection of CIN2+ or CIN3+.

	Dual-stained cytology		Pap cytology		HPV16/18 typing			
	Positive, *n/N*	Estimate (95% CI)	Positive, *n/N*	Estimate (95% CI)	Positive, *n/N*	Estimate (95% CI)	*P*[a]	*P*'[b]
Detection of CIN2+ ($N = 126$)								
Sensitivity	80/126	63.49% (54.4%–71.9%)	78/126	61.90% (52.8%–70.4%)	78/126	61.90% (52.8%–70.4%)	0.832	0.897
Specificity	614/720	85.28% (82.5%–87.8%)	576/720	80.00% (76.9%–82.9%)	521/720	72.36% (68.9%–75.6%)	<0.001	<0.001
PPV	80/186	43.01% (35.8%–50.5%)	78/222	35.14% (28.9%–41.8%)	78/277	28.16% (22.9%–33.8%)	0.001	<0.001
NPV	614/660	93.03% (90.8%–94.9%)	576/624	92.31% (89.9%–94.3%)	521/569	91.56% (89.0%–93.8%)	<0.001	<0.001
Detection of CIN3+ ($N = 116$)								
Sensitivity	75/116	64.66% (55.2%–73.3%)	73/116	62.93% (53.5%–71.7%)	73/116	62.93% (53.5%–71.7%)	0.824	0.892
Specificity	619/730	84.79% (82.0%–87.3%)	581/730	79.59% (76.5%–82.5%)	526/730	72.05% (68.6%–75.3%)	<0.001	<0.001
PPV	75/186	40.32% (33.2%–47.7%)	73/222	32.88% (26.7%–39.5%)	73/277	26.35% (21.3%–32.0%)	0.002	<0.001
NPV	619/660	93.79% (91.7%–95.5%)	581/624	93.11% (90.8%–95.0%)	526/569	92.44% (90.0%–94.5%)	<0.001	<0.001

Abbreviation: CI, confidence interval.
[a]*P* value indicates the comparison between dual-stained cytology and Pap cytology.
[b]*P*' value indicates the comparison between dual-stained cytology and HPV16/18 typing.

Table 3. Performance of combing HPV16/18 genotyping with dual-stained cytology versus with Pap cytology in HPV-positive women.

	HPV16/18 to colposcopy and triage 12 other HR-HPV(+) with dual-stained cytology		HPV16/18 to colposcopy and triage 12 other HR-HPV(+) with Pap cytology		
	Positive, *n/N*	Estimate (95% CI)	Positive, *n/N*	Estimate (95% CI)	*P*
Detection of CIN2+ ($N = 126$)					
Sensitivity	109/126	86.51% (79.3%–91.9%)	108/126	85.71% (78.4%–91.3%)	1.000
Specificity	450/720	62.50% (58.8%–66.0%)	418/720	58.06% (54.4%–61.7%)	<0.001
PPV	109/379	28.76% (24.2%–33.6%)	108/410	26.34% (22.1%–30.9%)	0.004
NPV	450/467	96.36% (94.2%–97.8%)	418/436	95.87% (93.6%–97.5%)	<0.001
Detection of CIN3+ ($N = 116$)					
Sensitivity	101/116	87.07% (79.6%–92.6%)	100/116	86.21% (78.6%–91.9%)	1.000
Specificity	452/730	61.92% (58.3%–65.5%)	420/730	57.53% (53.9%–61.2%)	<0.001
PPV	101/379	26.65% (22.3%–31.4%)	100/410	24.39% (20.3%–28.8%)	0.006
NPV	452/467	96.79% (94.8%–98.2%)	420/436	96.33% (94.1%–97.9%)	<0.001

Note: *P* value indicates the comparison between dual-stained cytology and HPV16/18 typing.
Abbreviations: CI, confidence interval; HR-HPV, high-risk human papillomavirus.

HPV16/18 genotyping with the dual-stained cytology needed a similar number of colposcopies detected per CIN2+ or CIN3+ ($n = 3.48$ for CIN2+, $n = 3.75$ for CIN3+), as compared with the combination strategy with Pap cytology, although the specificity was significantly higher (**Tables 3** and **4**).

Discussion

HPV testing has been approved for primary cervical cancer screening. However, HPV testing doubles the screen-positive population referral to colposcopy as compared with cytology-based screening (3). Finding effective triage strategies that can augment the specificity of the primary screening tests and reduce the referral to colposcopy for HPV-positive women remains a critical issue (15).

In our study, we first evaluated the positivity of dual stain among the HPV-positive women, which significantly increased with increasing severity of Pap cytology and the grade of the lesions (**Table 1**). However, the positivity of dual stain was unexpectedly low in cancer (5/12, 41.67%), probably on account of the necrosis of cancer tissues, which may confuse and influence the accuracy of results. Dual-stained cytology showed a lower positivity rate compared with Pap cytology (21.99% vs. 26.24%, $P = 0.041$; **Table 1**), and in clinical practice this could signify a lower referral to colposcopy (**Table 4**).

We also evaluated the clinical performance of p16/Ki-67 dual-stained cytology as compared with Pap cytology for the detection of CIN2+ or CIN3+. For the detection of CIN2+, the specificity of the dual-stained cytology was significantly higher than that of Pap cytology (85.28% vs. 80.00%, $P < 0.001$) while the sensitivity of dual-stained cytology remained comparable with Pap cytology (63.49% vs. 61.90%, $P > 0.05$; **Table 2**). Similar patterns were observed for the detection of CIN3+. These findings are of vital importance to the developing countries especially China, where conducting Pap cytology has faced many challenges. For example, experienced cytotechnologist and standardized professional training are scarce. Evaluation and quality control system are also far from perfect. In addition, Pap cytology is highly subjective, which results in considerable interlaboratory variation (16). Our study has shown dual-stained cytology with objective and excellent reproduction, could achieve similar or even better performance as compared with Pap cytology. Furthermore, recent research has also evaluated the longitudinal performance of p16/Ki-67 dual-stained cytology for triaging HPV-positive women and concluded that dual-stained cytology provides better long-term risk stratification than cytology over 5 years (17). These data have shown that dual-stained cytology test might be superior to Pap cytology as the "second line" triage test for HPV-positive women in China. HPV16/18 genotyping alone had the lowest sensitivity and specificity as a single triage. However, one practical advantage is that it occurs concurrently with the primary screening test.

Our findings are comparable with the study by Wentzensen and colleagues who showed that dual-stained cytology has an increased specificity (58.9% vs. 49.6%, $P < 0.001$) with similar sensitivity (83.4% vs. 76.6%, $P > 0.05$) for CIN2+ detection, compared with cytology in

Table 4. Detection of CIN2+ or CIN3+ versus colposcopies needed of different triage strategies.

	Number of colposcopies	Number of CIN2+ detected	Number of CIN3+ detected	Number of colposcopies per CIN2+ detected	Number of colposcopies per CIN3+ detected
Triage all HPV(+) with dual-stained cytology	186	80/126	75/116	2.33	2.48
Triage all HPV(+) with Pap cytology	222	78/126	73/116	2.85	3.04
Triage all HPV(+) with HPV 16/18 type	277	78/126	73/116	3.55	3.79
HPV16/18 to colposcopy and triage 12 other HR-HPV(+) with dual-stained cytology	379	109/126	101/116	3.48	3.75
HPV16/18 to colposcopy and triage 12 other HR-HPV(+) with Pap cytology	410	108/126	100/116	3.80	4.1

Abbreviation: HR-HPV, high-risk human papillomavirus.

the triage of 1,509 HPV-positive women in Kaiser Permanente Northern California (KPNC; ref. 9). However, the sensitivity of cytology at KPNC was much higher than ours, which may ascribe to the methods used for Pap cytology at KPNC. This was also confirmed by Ebisch and colleagues who showed dual-stained cytology would be the most reliable strategy in triage of HPV-positive women with an increased specificity (61% vs. 49%, $P < 0.05$) and similar sensitivity (92% vs. 93%, $P > 0.05$) compared with Pap cytology for CIN3+ detection (10). Our study results show deviation with the findings of the ATHENA trial, which showed dual-stained cytology was significantly more sensitive than Pap cytology (70.3% vs. 51.8%, $P < 0.001$) for triaging HPV-positive women, whereas specificity was comparable (75.6% vs.76.1%, $P > 0.05$; ref. 11). Several reasons may account for the differences in the utility of cervical cytology among different trials, and the most primary one is the highly subjective Pap cytology, which may lead to interlaboratory variation. Moreover, the liquid-based cervical specimen in KPNC were evaluated using computer-assisted imaging followed by cytotechnologist review with the knowledge of HPV test results and HPV status when reporting Pap cytology results, leading to a higher sensitivity at a compromise of specificity (18). The positivity rate of dual stain in our study (21.99%) was similar to that of the ATHENA trial (28.4%), while being much lower than that of the KPNC trial (46.0%).

The triage utility for HPV-positive women was also assessed by combining HPV16/18 genotyping with the dual-stained cytology. The dual-stained cytology, combined with HPV16/18 genotyping, showed a significantly higher sensitivity as compared with other triage strategies (86.51% for CIN2+, 87.07% for CIN3+) and maintained a relatively moderate specificity (62.50%) compared with other clinical trials such as KPNC and ATHENA trials. Furthermore, HPV16/18 genotyping combined with dual-stained cytology showed a higher specificity (62.50% vs. 58.06%, $P < 0.001$), PPV (28.76% vs. 26.34%, $P = 0.004$), and NPV (96.36% vs. 95.87%, $P < 0.001$) as compared with combination with Pap cytology, whereas the sensitivity of both combination tests were identical for CIN2+ detection. Similar patterns were observed for CIN3+ detection (**Table 3**).

Our study has several strengths. We have evaluated a large cohort with uniform and well-organized screening and management procedures through a multicenter study in China. Furthermore, all included women had a definite pathologic diagnosis through colposcopy. Limitations also existed. We used the second cytology specimen for the dual-stained cytology, half of which was used for Pap cytology, and the dual-stained slides were processed and evaluated in a central laboratory. Furthermore, there is a relatively low proportion of CIN2 (10/126, 7.94%) compared with CIN3 (104/126, 82.54%) in our study. This may ascribe to the subjective diagnosis of CIN2, which is difficult to distinguish from CIN3 in individual cases. The skewed distribution of CIN2 may influence the performance of dual stain in triaging HPV-positive women.

In conclusion, we have assessed the clinical performance of p16/Ki-67 dual-stained cytology for CIN2+ or CIN3+ detection in triaging HPV-positive women. Dual-stained cytology, either alone or in combination with HPV16/18 genotyping, represents a promising approach as a specific and efficient triage strategy for HPV-positive women.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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