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Triage Value of Cervical Exfoliated Cell DNA Ploidy Analysis in Cervical High-Risk Human Papillomavirus–Positive Women

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Objective: This study aimed to investigate the triage value obtained in DNA ploidy analysis of cervical exfoliated cells in women with high-risk human papillomavirus (HR-HPV)-positive status in the primary screening of cervical cancer.

Methods: The authors selected 3,000 HR-HPV–positive women for cervical exfoliated cell sampling and conducted DNA ploidy analysis, liquid-based cytology (LBC), colposcopy, and cervical biopsy. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of high-grade squamous intraepithelial lesion (HSIL)-positive detection between DNA ploidy analysis and LBC were compared according to histopathology diagnosis as the golden criteria, and the efficacy of predicting HSIL-positive immediate risk was evaluated.

Results: A total of 2,892 HR-HPV–positive women were enrolled in the investigation. For HSIL+ women, the DNA ploidy group showed a significantly higher sensitivity (CIN2+: 79.21% vs 65.35%, $p = .022$; CIN3+: 81.48% vs 70.37%, $p = .013$), lower specificity (CIN2+: 85.00% vs 96.59%, $p < .001$; CIN3+: 84.14% vs 93.41%, $p < .001$), and lower PPV (CIN2+: 16.23% vs 29.33%, $p = .001$; CIN3+: 8.92% vs 16.89%, $p = .002$) compared with the LBC group, whereas the NPV showed no significant difference. Compared with LBC alone in diagnosing HSIL, DNA ploidy combined with LBC showed higher specificity (CIN2+: 99.21% vs 96.59%, $p = .003$; CIN3+: 96.48% vs 93.41%, $p < .001$) and higher PPV (CIN2+: 41.35% vs 29.33%, $p = .022$; CIN3+: 24.81% vs 16.89%, $p = .028$), whereas no significant difference was observed in the sensitivity (CIN2+: 54.46% vs 65.35%, $p = .063$; CIN3+: 61.11% vs 70.37%, $p = .221$) and NPV ($p > .05$). Among the HR-HPV–positive women positive for DNA ploidy, the imminent risk of CIN2+ and CIN3+ were 15.62% and 8.92%, respectively, above the threshold for the colposcopy positive rate. Among the positive cases both for DNA ploidy and the LBC result of negative for intraepithelial lesion or malignancy, the immediate risk of CIN3+ was 3.31%, below the threshold for colposcopy positive rate. Besides, for women with LBC result of ASC-US and above, the immediate risk of CIN3+ was greater than 4%.

Conclusions: The DNA ploidy analysis can be used as an effective triage method for HR-HPV–positive women during the primary screening of cervical cancer, although it can provide higher specificity when combined with LBC and reduce the referral rate for colposcopy.

Key Words: DNA ploidy, liquid-based cytology, high-risk, human papillomavirus, colposcopy, cervical intraepithelial neoplasia, cervical cancer

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The study was approved by the ethics committee of Huzhou Maternity & Child Health Care Hospital (Ethical Committee number 2022-J-077 [Approval date: August 22, 2022] Chairperson: Pingya He).

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Cervical cancer is the fourth most common cancer and the fourth leading cause of cancer-related deaths among women worldwide.[1] There are more than 100,000 new cases of cervical cancer in China every year.[2] Fortunately, cervical cancer can be prevented by effective screening and active treatment.[3] Developed countries and regions have demonstrated great success in the reduction of cervical cancer-related morbidity and mortality by effective screening programs.[1] Because China has a relatively high proportion of the total incidence of global cervical cancer cases, effective screening programs are particularly important for China, which has a vast land area with large population and a relatively low immunization coverage rate for human papillomavirus (HPV) vaccine.[4]

Currently, 3 programs for cervical cancer primary screening are recommended worldwide: liquid-based cytology (LBC) primary screening, high-risk (HR)-HPV primary screening, and combined LBC + HR-HPV screening.[5] Human papillomavirus testing has high sensitivity, whereas LBC is commonly used internationally for triage screening, but this technique faces substantial challenges in countries and regions lacking cytopathology doctors with high expertise.[6–10] In this way, HR-HPV primary screening has become the main method of large-scale systematic screening for women in China.[11] Although cytology has high specificity, its sensitivity is relatively low.[12] Thus, the results are limited by the subjective experience of cytopathologists and the high time requirement for training a qualified cytology physician. Therefore, there is a pressing need to find a more ideal triage method to reduce the morbidity of cervical cancer and achieve the global strategic goal of eliminating this type of cancer.[13]

At present, DNA ploidy analysis technology has been able to realize the automation of specimen test and diagnosis and is widely used in the auxiliary diagnosis of various tumors such as endometrial carcinoma and pulmonary carcinoma.[14,15] Studies have shown that abnormalities in the content of chromosomes and DNA in the nucleus occur significantly earlier than pathological changes in cell morphology during the occurrence and progression of cervical lesions. Compared with morphological LBC screening technology, the detection ability of high-grade cervical lesions by DNA ploidy analysis may provide more benefits.[16–18]

Nevertheless, there is still no clear consensus on the value of DNA ploidy analysis in the triage of HR-HPV–positive women, and large-sample studies are still rare. Thus, the purpose of this study was to compare the triage efficacy of DNA ploidy analysis and LBC in HR-HPV–positive women in the primary screening of cervical cancer, to explore the significance of triage with DNA ploidy analysis alone or in combination with cytology, as well as the role of DNA ploidy analysis in the immediate risk evaluation of cervical HSIL+.

MATERIALS AND METHODS

Patient Characteristics

A total of 3,000 HR-HPV–positive women who underwent opportunistic screening for cervical cancer in our hospital from June 2018 to June 2021 were selected. Patients were eligible if

they met the following criteria: (1) women aged 25–65 years, with sexual experience and (2) HR-HPV–positive in the primary screening of cervical cancer (by Aptima HPV E6/E7 mRNA test [Gen-Probe, San Diego, CA], hybrid capture 2 [HC2] HPV test [Qiagen, Germantown, MD], Cervista HPV test [Hologic, Marlborough, MA], and Cobas 4800 HPV test [Roche Molecular Systems, South Branchburg, NJ]). The exclusion criteria were as follows: (1) pregnant women, (2) patients with severe immunodeficiency, (3) patients with a personal history of genital tract malignancies, (4) patients with untreated genital tract infections, and (5) patients unwilling to participate in the study. The cervical exfoliated cells during the nonmenstrual period were collected to make liquid-based, thin-layer cell smears for LBC test and DNA ploidy analysis, respectively. After giving their full informed consent, all subjects underwent colposcopy and cervical biopsy for histopathological diagnosis. This study was approved by the ethics committee of our hospital.

Methods

Liquid-Based Cytology. The Thinprep Cytology Test method was used for LBC. The cytological diagnoses of the LBC slices were made by 2 experienced pathologists in our hospital according to the Bethesda System 2014[19]: negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (SCC), and atypical glandular cells-not otherwise specified (AGC-NOS). Cytological test results of ASC-US and above were considered abnormal.

DNA Ploidy Analysis of Cervical Cells. After Feulgen staining, the specimens were scanned by the Motic BA600 (MOTIC CHINA GROUP CO., LTD, Xiamen, China) automatic high-resolution cellular DNA image analysis system. The system analyzed multiple parameters of each sample nucleus and automatically completed cell counting and classification according to different characteristic parameters. The DNA content in the cells was represented by "c". A normal single cell is a diploid cell in the G0/G1 phase of the cell cycle and is represented by "2c". When the cell enters the G2/M phase, it is a tetraploid and is represented by "4c". When the cell has abnormal division, that is, when the DNA ploidy is 5c or greater, it is judged as an abnormal ploidy cell; with the "5c" abnormal ploidy cell, a positive judgment is made.[20]

Histopathological Diagnosis. According to the histopathological diagnostic criteria of the 2020 World Health Organization classification of female genital tumors,[21] cervical lesions are divided into normal or inflammatory changes (normal), low-grade squamous intraepithelial lesions (LSIL/CIN1), high-grade squamous intraepithelial lesions (HSIL/CIN2–3), and carcinomas (SCC, adenocarcinoma, and adenosquamous carcinoma [ASC]). The histopathological slices were evaluated by 2 independent experienced gynecological pathologists. When the 2 pathologists disagreed, a third senior gynecological pathologist made the final decision. A histopathological diagnosis of HSIL and above was regarded as positive.

Evaluation Indicators. The detection sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used to analyze and compare the diagnostic performance of each detection method for detecting HSIL+ (CIN2+ and CIN3+), and the percentage of existing lesions with different degrees at the detection were used to calculate the immediate risk of HSIL+ (CIN2+ and CIN3+).

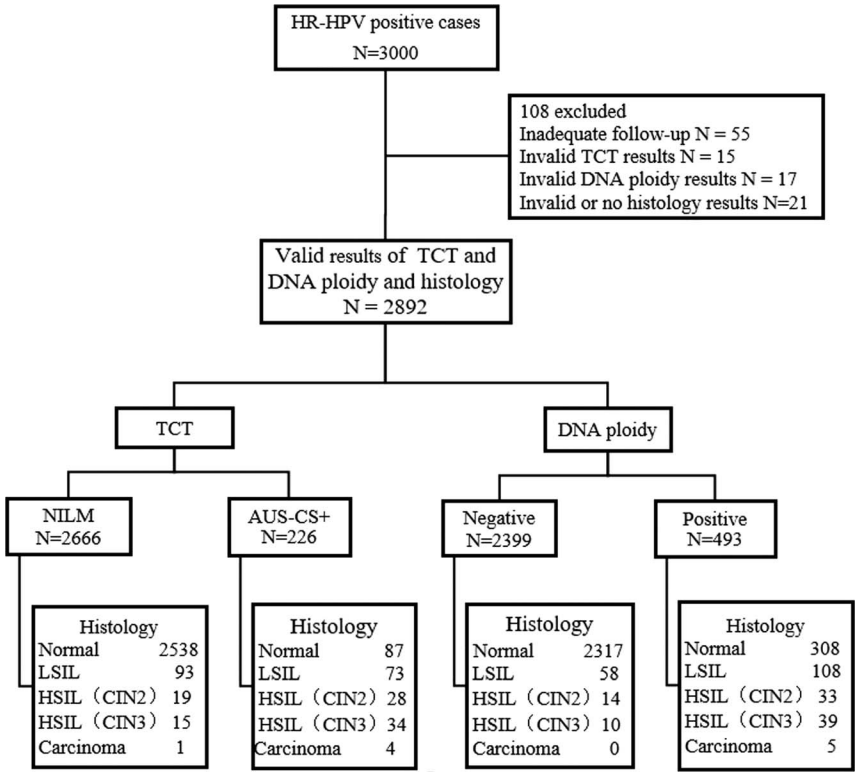

FIGURE 1. Flowchart of a comparative study of different triage methods for women with HR-HPV. TCT indicates Thinprep Cytology Test.

TABLE 1. Distribution of Results of TCT, DNA Ploidy Analysis and Histopathological Test

		TCT						DNA ploidy	
Histology	**Total**	**NILM**	**ASC-US**	**LSIL**	**ASC-H**	**HSIL**	**AGC-NOS**	**Negative**	**Positive**
Normal	2,625	2,538	57	28	1	1	0	2,317	308
LSIL	166	93	23	46	3	0	1	58	108
HSIL/CIN2	47	19	11	10	1	6	0	14	33
HSIL/CIN3	49	15	4	8	4	18	0	10	39
Carcinoma	5	1	1	0	0	3	0	0	5
Total	2,892	2,666	96	92	9	28	1	2,399	493

TCT indicates Thinprep Cytology Test.

Statistical Analysis

Data analysis was performed by SPSS 22.0 statistical software, and descriptive statistical methods were used to calculate the sensitivity, specificity, PPV, and NPV to describe the statistical indicators. A value of $p < .05$ was considered as a statistically significant difference.

RESULTS

Baseline Data

Among the 3,000 women, a total of 2,892 HR-HPV–positive women (Figure 1) were finally included in the study. The average age was 39.3 ± 9.7 years. Women with positive HR-HPV test results were subjected to LBC and DNA ploidy analysis at the same time, while they received colposcopy and cervical biopsy for histopathological confirmation. Among them, 101 cases were histopathologically diagnosed as HSIL+ (CIN2+), including 5 cases of cervical cancer, 166 cases of LSIL, and 2,625 cases of no abnormal lesions by histopathology.

Distribution of Different Triage Detection and the Histopathological Results

Among the 2,892 cases included in the final analysis, there were 2,666 cases with no abnormal lesions, 96 cases with ASC-US, 9 cases with ASC-H, 92 cases with LISL, 28 cases with HISL, and 1 case with AGC-NOS, as determined by cytological diagnosis. The DNA ploidy analysis showed no abnormal ploidy in 2,399 cases and abnormal ploidy in 493 cases (Table 1). The overall abnormal rate of LBC was 7.81%, and the overall positive rate of DNA ploidy analysis was 17.05%. The false-negative rate of DNA ploidy was 3.42%, which was significantly lower than the 4.80% false-negative rate of LBC; $p = .014$.

Comparison of the Efficacy of DNA Ploidy and LBC for Detecting HSIL+

In the triage test, the sensitivity of DNA ploidy analysis in detecting CIN2+ was significantly higher than that of LBC (79.21% vs 65.35%, $p = .022$), whereas the specificity (85.00% vs 96.59%, $p < .001$) as well as the PPV (16.23% vs 29.33%, $p = .001$) were significantly lower. There was no significant difference in the NPV between the 2 (99.00% vs 98.57%, $p = .171$). When detecting CIN3+, the sensitivity of DNA ploidy was significantly higher than that of LBC (81.48% vs 70.37%, $p = .013$), whereas the specificity (84.14% vs 93.41%, $p < .001$) and the PPV (8.92% vs 16.89%, $p = .002$) were significantly lower. There was no significant difference in the NPV (99.54% vs 99.40%, $p = .461$) (Table 2 and 3).

Comparison of the Efficacy of DNA Ploidy Combined With LBC and LBC Alone for the Triage Test of HSIL+

In the triage test, when DNA ploidy was combined with LBC, the specificity in detecting CIN2+ (99.21% vs 96.59%, $p = .003$) and the PPV (41.35% vs 29.22%, $p = .022$) were significantly higher than that for LBC alone, whereas the sensitivity (54.46% vs 65.35%, $p = .063$) and the NPV (98.30% vs 98.57%, $p = .196$) showed no significant difference. When detecting CIN3+, the specificity (96.48% vs 93.41%, $p < .001$) and the PPV (24.81% vs 16.89%, $p = .028$) of the combined triage test was significantly higher than that for LBC alone, whereas the sensitivity (61.11% vs 70.37%, $p = .221$) and the NPV (99.24% vs 99.40%, $p = .383$) showed no significant difference (Table 4 and 5).

TABLE 2. Comparison of the Efficacy of TCT and DNA Ploidy Analysis for the Detection of CIN2+

	TCT	DNA ploidy	
Histology	**% (95% CI)**	**% (95% CI)**	***p***
CIN2 + ($n = 101$)			
Sensitivity	65.35 (55.64–73.93)	79.21 (70.23–86.05)	0.022
Specificity	96.59 (95.28–97.53)	85.00 (82.88–86.96)	<0.001
PPV	29.33 (23.76–35.60)	16.23 (13.23–19.75)	0.001
NPV	98.57 (97.55–99.12)	99.00 (98.16–99.49)	0.171

TABLE 3. Comparison of the Efficacy of DNA Ploidy Analysis and TCT for the Detection of CIN3+

	TCT	DNA ploidy	
Histology	**% (95% CI)**	**% (95% CI)**	***p***
CIN3 + ($n = 54$)			
Sensitivity	70.37 (57.10–80.93)	81.48 (68.97–89.81)	0.013
Specificity	93.41 (91.35–95.12)	84.14 (83.54–87.47)	<0.001
PPV	16.89 (12.53–22.36)	8.92 (6.70–11.79)	0.002
NPV	99.40 (98.43–99.48)	99.54 (98.41–99.58)	0.461

TABLE 4. Comparison of the Efficacy of TCT Alone and DNA Ploidy Analysis Combined With TCT in Triage Test for the Diagnosis of CIN2+

	TCT	DNA ploidy + TCT	
Histology	**% (95% CI)**	**% (95% CI)**	***p***
CIN2 + (n = 101)			
Sensitivity	65.35 (55.64–73.93)	54.46 (44.76–63.83)	0.063
Specificity	96.59 (95.28–97.53)	99.21 (96.50–97.77)	0.003
PPV	29.33 (23.76–35.60)	41.35 (32.99–50.23)	0.022
NPV	98.57 (97.55–99.12)	98.30 (97.72–98.73)	0.196

Immediate Risk Value of HSIL+ in HPV-Positive Women Evaluated by DNA Ploidy Alone

In HR-HPV–positive women with negative DNA ploidy analysis, regardless of the LBC results, the overall immediate risks of CIN2+ and CIN3+ were relatively low, at 1.00% and 0.42%, respectively. When the DNA ploidy analysis was positive, the immediate risk of CIN2+ and CIN3+ in HPV-positive women significantly increased, at 15.62% and 8.92%, respectively (Table 6).

Immediate Risk Value of CIN2+ Evaluated by DNA Ploidy Combined With LBC

Paired combinations of different results of DNA ploidy analysis and LBC were used to calculate the different immediate risk values of CIN2+ and CIN3+. When there was no abnormality in either DNA ploidy and LBC, the immediate risk values of CIN2 + and CIN3+ were relatively low at 0.48% and 0.04%, respectively. In the cases of positive DNA ploidy analysis, the immediate risk values of CIN2+ and CIN3+ were 6.63% and 3.31% if the LBC was NILM, and the immediate risk was higher than 10% if the LBC was abnormal. When the DNA ploidy was normal, the immediate risk of CIN2+ and CIN3+ reached 85.71% if the LBC was HSIL. When the DNA ploidy was normal but the LBC showed LSIL, ASC-H, and AGC-NOS, the corresponding risk values could not be calculated because no corresponding case was found (Table 7).

DISCUSSION

Cervical cancer is a common malignancy that seriously threatens women's health. China and India together contribute to more than one third of the global cervical cancer cases and deaths.[1,22] High-risk HPV infection is a necessary precondition for cervical SCC. At the same time, it is usually temporary, with the infection clearing up in approximately 80% of cases within 24 months, whereas the development of CIN and cervical cancer is almost always accompanied by persistent HR-HPV infection.[23,24] Human papillomavirus testing has high sensitivity, and HR-HPV detection has been proven feasible as a primary screening protocol for cervical cancer in China,[25–27] although it has a high false-positive rate that may easily lead to unnecessary colposcopy. Meanwhile, LBC has low sensitivity and is influenced by subjective factors, which necessitates the development of a triage method to retain the high sensitivity of HPV detection and simultaneously improve its screening specificity, to effectively reduce unnecessary colposcopy referrals.

TABLE 5. Comparison of the Efficacy of TCT Alone and DNA Ploidy Analysis Combined With TCT in Triage Test for the Diagnosis of CIN3+

	TCT	DNA ploidy + TCT	
Histology	**% (95% CI)**	**% (95% CI)**	***p***
CIN3 + (n = 54)			
Sensitivity	70.37 (57.10–80.93)	61.11 (47.77–72.98)	0.221
Specificity	93.41 (91.35–95.12)	96.48 (95.71–97.11)	<0.001
PPV	16.89 (12.53–22.36)	24.81 (17.92–33.19)	0.028
NPV	99.40 (98.43–99.48)	99.24 (98.82–99.52)	0.383

TABLE 6. Immediate Risks of HSIL+ in HR-HPV Positive Women Predicted by DNA Ploidy Analysis Alone

DNA ploidy	N	%	CIN2 + cases	CIN2+ immediate risk, %	CIN3 + cases	CIN3+ immediate risk, %
Negative	2,399	82.95	24	1.00	10	0.42
Positive	493	17.05	77	15.62	44	8.92
Total	2,892	100.00	101	3.49	54	1.87

Studies have established that the change in cellular DNA ploidy content occurs earlier than the morphological change, which is an important indicator of early malignant lesions.[28] Our results showed that in HR-HPV–positive women, whether CIN2 + or CIN3+, the sensitivity of DNA ploidy analysis in the triage test was higher than that of LBC, with a statistically significant difference, which is in agreement with the results of studies by other scholars.[29,30] Our results also showed a low false-negative rate of DNA ploidy of 3.42%, significantly lower than 4.80% for LBC (p = .014). Thus, we further compared the efficacy of DNA ploidy analysis combined with LBC and LBC triage test alone in HR-HPV–positive women. The results showed that the specificity and PPV of DNA ploidy combined with LBC were significantly higher than those of LBC alone in HSIL+ detection. Our results showed that according to the threshold in the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines, 7.81% of HR-HPV–positive women needed to be referred to colposcopy for examination or treatment when cytological triage was used alone, whereas this rate was 17.05% for DNA ploidy triage alone. If combined screening was used, their colposcopy referral rate was 6.05%. These data indicate that HR-HPV–positive women can be triaged by a combined test of DNA ploidy analysis and LBC, and HSIL+ cases can be detected more specifically, which is helpful for the efficient reduction of unnecessary colposcopy referrals.

According to the 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors, we tried to calculate the immediate risk of HSIL+ in HR-HPV–positive women predicted by DNA ploidy analysis alone, and the immediate risk values of HSIL+ predicted by different combinations of DNA ploidy analysis and LBC. We found that in HR-HPV–positive women, when the DNA ploidy analysis was positive, the immediate risk was 14.29% for CIN3+, reaching the clinical action threshold for colposcopy referral proposed by ASCCP in 2019.[8] These data suggest that when HR-HPV is positive in clinical practice, referral for colposcopy should be recommended if DNA ploidy analysis is positive even without an LBC result. In the case of positive DNA ploidy value, if the LBC result was NILM, CIN3+ had an immediate risk of 3.31%, lower than the 4% threshold for colposcopy referral proposed by ASCCP in 2019, and follow-ups could be recommended. Our findings suggest that combined triage can

TABLE 7. Immediate Risks of HSIL+ in HR-HPV–Positive Women Predicted by Different Combinations of TCT and DNA Ploidy Analysis

TCT	DNA ploidy	N	%	CIN2+ cases	CIN2+ immediate risk, %	CIN3+ cases	CIN3+ immediate risk, %
NILM	Positive	362	12.52	24	6.63	12	3.31
ASCUS	Positive	45	1.56	13	28.89	5	11.11
LSIL	Positive	58	2.01	14	24.14	8	13.79
ASC-H	Positive	7	0.24	5	71.43	4	57.14
HSIL	Positive	21	0.73	21	100.00	15	71.43
	Total	493	17.05	77	15.62	44	8.92
NILM	Negative	2,301	79.56	11	0.48	1	0.04
ASCUS	Negative	54	1.87	3	5.56	2	3.70
LSIL	Negative	34	1.18	4	11.76	0	0.00
ASC-H	Negative	2	0.07	0	0.00	0	0.00
HSIL	Negative	7	0.24	6	85.71	6	85.71
AGC-NOS	Negative	1	0.03	0	0.00	0	0.00
	Total	2,399	82.95	24	1.00	10	0.42
Total		2,892	100.00	101	3.49	54	1.87

effectively reduce the number of referrals for colposcopy. When the LBC results are ASC-US or above, the immediate risks of CIN2+ and CIN3+ were both higher than 4%, and the immediate risk values of CIN3+ were 11.11% for ASC-US, 13.79% for LSIL, 57.14% for ASC-H, and 71.43% for HSIL. These values were all higher than the results of Didem Egemen et al.,[31] who predicted the immediate risks of CIN3+ to be 5.4% for ASC-US, 5.0% for LSIL, 22% for ASC-H, and 44% for HSIL, based on LBC alone. This means that when the HR-HPV primary screening is positive, the risk of cervical precancer and cervical cancer is higher when DNA ploidy combined with LBC is positive compared with when LBC alone is abnormal, showing that DNA ploidy test combined with LBC can improve the specificity and PPV of screening. In the case of negative DNA ploidy analysis, when the LBC result was NILM, routine follow-up could be performed. On the other hand, when the LBC result was ASC-US, all cases were CIN3+, and the immediate risk of CIN3+ was 5.56%. When the LBC result was HSIL, all cases were CIN3+, and the immediate risk of CIN3+ was 85.71%. Based on our findings and previous related studies, it is suggested that the risk of HSIL+ is relatively high when the results of LBC and DNA ploidy analysis are both abnormal for HR-HPV–positive women in primary screening; thus we should pay more attention and deal with it.

However, this was a feasibility study on DNA ploidy analysis in cervical cancer screening. Our research also has some shortcomings. For example, when calculating the predictive HSIL+ risks by different combinations, the results cannot be obtained due to the lack of corresponding samples for some combinations, which may be related to the small sample size. In addition, the 5-year HSIL+ risk could not be calculated and predicted due to the limited research period, which prompts future studies with larger sample size and longer follow-up times to further confirm its value in long-term prediction. Moreover, we have not conducted further cost-efficiency comparisons. In addition, not all HR-HPV–positive women undergo HPV genotyping testing. Next, we plan to conduct HPV genotyping testing for all HR-HPV–positive women according to the 2019 ASCCP guidelines, exploring the feasibility and cost-effectiveness of DNA ploidy analysis in stratified studies of HR-HPV non-16/18 positive women.

In conclusion, DNA ploidy analysis can be used as an effective triage method for HR-HPV–positive women during the primary screening of cervical cancer, although it can provide higher specificity when combined with LBC and reduce the referral rate for colposcopy. This technology is feasible in the triage of cervical cancer screening. Meanwhile, to better guide subsequent examinations or treatments according to corresponding tests, the immediate risk of HSIL+ can be predicted, and the effective management of women at high risk of cervical cancer and precancerous lesions can be strengthened.

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