Original Article

Triage of women with positive HPV: comparing DNA ploidy analysis with HPV 16/18 genotyping and cervical cytology

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Background: Cervical cancer screening primarily uses the human papillomavirus (HPV) test with partial genotyping (HPV 16/18) and liquid-based cytology using ThinPrep cytology test (TCT) to triage women with a positive HPV test. Although quantitative DNA ploidy analysis has shown reliability, its integration into screening guidelines as a triage test, compared to partial genotyping and TCT, has not been fully established. The objective of the study is to evaluate the clinical utility of DNA ploidy analysis as a triage test for women with a positive HPV test in primary screening, comparing it to HPV 16/18 genotyping and TCT.

Methods: We retrospectively analyzed data from 335 women aged ≥18 years who participated in a cervical cancer screening program at Shanghai Ruijin Hospital and underwent triage using HPV 16/18, TCT, and DNA ploidy testing. The sensitivities and specificities of these methods, both individually and combined, were evaluated.

Results: The test showed sensitivities and specificities of 35.4% and 76.1% for HPV 16/18, 29.2% and 88.2% for TCT, and 93.8% and 92.7% for DNA ploidy, respectively. Combining these tests improved outcomes, with DNA ploidy plus HPV 16/18 genotyping showing enhanced sensitivity and high specificity. Notably, DNA ploidy alone identified high-grade squamous intraepithelial lesions (HSIL) and cervical cancer with a higher detection rate and lower positivity rate in triage than HPV 16/18 and TCT.

Conclusions: DNA ploidy analysis demonstrated superior specificity and sensitivity in the triage of women with positive HPV test results, offering a more effective alternative for detecting high-grade lesions and cervical cancer. These findings support the potential of integrating DNA ploidy into current cervical cancer screening protocols to enhance triage effectiveness and reduce unnecessary colposcopy referrals.

Keywords: Cervical cancer; high-grade squamous intraepithelial lesion (HSIL); DNA ploidy analysis

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Introduction

Cervical cancer remains a significant public health concern globally as it is the fourth most common cancer affecting women worldwide after breast, colorectal, and lung cancers. It accounts for approximately 660,000 new cases and 350,000 deaths annually (1). It arises from the cervix and is primarily driven by persistent infection with high-risk human papillomavirus (HPV). Early detection through effective screening programs, such as those utilizing HPV testing to detect oncogenic HPV DNA, has proven crucial for reducing both the incidence and mortality of cervical cancer. In 2019, China reported 109,759 new cases, underscoring the widespread impact of the disease (2). The shift toward HPV testing has enhanced the precision of screening programs compared to cytology-based methods

alone. This has been pivotal in identifying at-risk women earlier and more reliably, thereby allowing for timely interventions and management. Moreover, advances in HPV vaccination have begun to alter the landscape of cervical cancer incidence, promising a future decrease in its prevalence. Nonetheless, challenges remain, including the integration of these tools into coordinated screening efforts worldwide, particularly in regions where access to healthcare is limited and disease burden is the highest (3-5).

The risk of cervical cancer or precancerous lesions is closely associated with the type and duration of HPV infection. Persistent infection with high-risk HPV types is responsible for more than 99% of the cervical cancer cases. HPV 16/18 are of particular concern and account for more than 70% of all cervical cancer cases (6). Cervical cancer progression is characterized by a series of cellular and molecular alterations (7). Cervical cytology using the ThinPrep cytology test (TCT) aids in the collection of exfoliated cells from the cervix using a liquid-based method and an automated processing system, facilitating cytological examination to detect potential cancerous changes. Currently, the integrated use of HPV testing and the TCT constitutes the foundation of cervical cancer screening strategies (8).

The updated guidelines recommend that negative HPV screening results be followed up at intervals of 5 years, reflecting the low 5-year risk of cervical intraepithelial neoplasia (CIN) III+ for HPV-negative and co-screening-negative cases, which are recorded at 0.14% [95% confidence interval (CI), 0.13–0.15%] and 0.12% (95% CI, 0.12–0.13%), respectively (9). These risks are below the threshold of 0.15%. However, the primary challenge in cervical screening is not the risk of CIN III+ among women with a negative HPV DNA test but the test's low specificity, which necessitates further triage. Current strategies, such as partial genotyping for HPV 16/18 and cytological triage for women with non-HPV 16/18 positive tests, are hampered by the limited sensitivity and specificity of genotyping and the subjective and error-prone nature of cytological assessments. Therefore, it is crucial to develop more accurate and reliable triage methods to effectively identify women at significant risk after a positive HPV DNA test, thereby enhancing the efficacy of cervical cancer screening programs.

At the molecular level, cervical carcinogenesis involves mutations in multiple genes, including the activation of oncogenes and the inactivation of tumor suppressor genes. Concurrently, alterations in the signaling pathways related to cell growth, apoptosis, and DNA damage repair disrupt the regulation of cell proliferation, facilitating the growth and survival of cancer cells. Quantitative DNA ploidy analysis, which detects abnormal DNA content in the nuclei of cervical epithelial cells, provides insight into the proliferative potential of tumor cells and has emerged as a valuable diagnostic tool for identifying cervical cancer and its precursors (10,11). Given the need for more objective biomarkers in the triage of women with a positive HPV test in primary screening, to better balance benefits and harms, this study aimed to evaluate the clinical utility of DNA ploidy analysis as a triage test for women with a positive HPV test in primary screening, comparing it to HPV 16/18 genotyping and TCT.

This study employed a retrospective cross-sectional design. We collected demographics, diagnoses, colposcopy

Highlight box

Key findings

- DNA ploidy analysis offers superior specificity and sensitivity in triage of women with a positive human papillomavirus (HPV) test compared to those offered by HPV 16/18 genotyping and ThinPrep cytology test (TCT).
- DNA ploidy's lower positivity rate in triage compared to other methods suggests that it could decrease unnecessary colposcopy referrals.

What is known and what is new?

- World Health Organization guidelines recommend HPV-DNA detection as the primary screening method for cervical cancer prevention in the general population of women, beginning at the age of 30 years, with regular testing every 5–10 years. For women with a positive HPV test, triage with partial genotyping (HPV 16/18) and cytological testing is recommended.
- Improving triage: this study revealed that DNA ploidy analysis offers superior specificity and sensitivity than HPV 16/18 genotyping and cytological testing.
- Resource optimization: DNA ploidy analysis could reduce unnecessary colposcopy referrals, suggesting its potential to optimize resource use in cervical cancer screening.

What is the implication, and what should change now?

- Enhancing triage accuracy: DNA ploidy analysis should be considered as an alternative to partial genotyping and cervical cytology in the triage of women with a positive HPV test in primary screening. This change could significantly improve the accuracy of detecting high-grade lesions and cervical cancer.
- Optimizing screening protocols: implementing DNA ploidy analysis as a standard triage tool could reduce unnecessary colposcopy referrals, thus optimizing resource use and potentially leading to better patient outcomes in cervical cancer prevention programs.

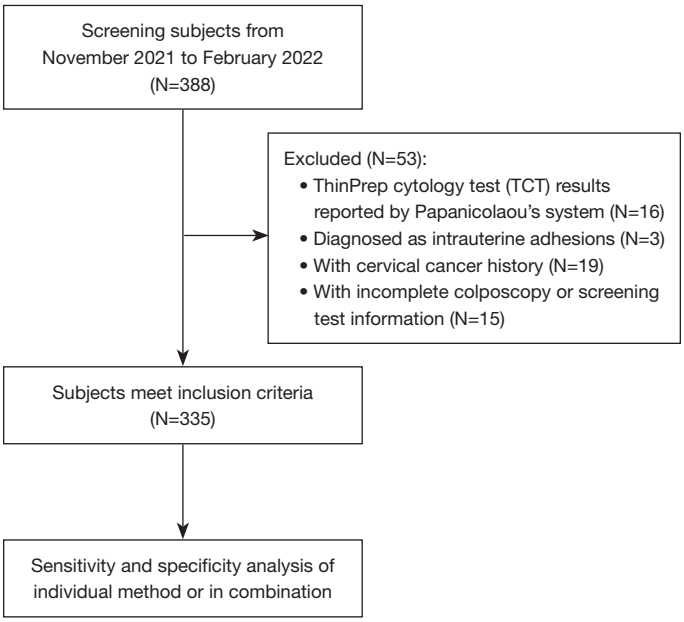

Figure 1 Flow chart of the screening process.

and biopsy results, and other laboratory tests, including HPV, TCT, and DNA ploidy, which were performed as part of the cervical cancer or precancerous lesion screening program at Ruijin Hospital. The data collection period spanned from November 2021 to February 2022, as detailed in Table S1. Logistic regression was used to evaluate the association between screening tests and the detection rates of high-grade squamous intraepithelial lesions (HSIL) and cervical cancer, as confirmed using colposcopy and biopsy. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1455/rc).

Methods

This was a retrospective, cross-sectional study. Demographics, diagnosis, colposcopy, and other laboratory tests, including HPV, TCT, and DNA ploidy, which were used for cervical cancer or precancerous lesion screening of eligible women who visited Shanghai Ruijin Hospital between November 2021 and February 2022 for the cervical cancer screening program were collected (Table S1). The association between the screening tests and degree of cervical malignancy confirmed using colposcopy was evaluated using logistic regression. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective study was exempt from review by the Ethics Committee of Ruijin Hospital because this type of study is a non-human subject research, and the need for informed consent was waived.

Participants

Women aged ≥18 years who had visited or were in the periodic follow-up of the Department of Gynecology of Shanghai Ruijin Hospital, China for a cervical cancer screening program between November 1, 2021 and February 28, 2022 were retrospectively identified from the in-house electronic medical record system in June 2024. All participants underwent human-performed HPV testing, cervical ThinPrep liquid-based cytology testing, and cervical DNA ploidy analysis during recruitment. Patients whose TCT results were reported using the Papanicolaou system, were diagnosed with intrauterine adhesions, had a history of cervical cancer, or had incomplete colposcopy information were excluded from the analysis (*Figure 1*). The degree of malignancy of the cervical epithelial cells was detected using colposcopy. Cervical biopsy was considered the gold standard method for all participants. Information recorded at the date of the hospital visit, such as demographics including age, smoking history, menopausal status, number of pregnancies, abortion information, delivery information, screening test results, and colposcopy or biopsy results, was collected (Table S2). To minimize systematic errors induced

during testing, the same clinician was responsible for sample collection and the same technician was responsible for HPV, TCT, and DNA ploidy testing.

HPV testing

An endocervical brush was placed on the cervix and rotated 2–3 times to collect cervical tissue for HPV E6/E7 mRNA detection. The HPV test (Health Gene Technologies, Inc., Shanghai, China) utilizes multiplex polymerase chain reaction (PCR) and capillary electrophoresis to detect 25 different HPV types based on the length of specific amplification fragments. The high-risk HPV genotypes were 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. The low-risk HPV types were 6, 11, 42, 43, 44, 81, and 83. HPV 16/18 positivity was defined as the presence of 16 or 18 HPV genotypes.

TCT

Participant's endocervical brush sample was rinsed in a vial filled with 20 mL of PreservCyt® Solution (PreservCyt). The ThinPrep sample vials were processed using a ThinPrep 5000 Processor (Hologic, Inc., Shanghai, China). The cells were collected from the exterior surface of the membrane and transferred to a thin cell smear with a diameter of 2 cm. Smears were fixed with 95% alcohol, stained with xylene, and sealed. All smears were reviewed by a pathologist according to the Bethesda system. TCT diagnoses were divided into negative for intraepithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), HSIL, and squamous cell carcinomas (SCC). The TCT-positive outcomes were defined as ASCUS, LSIL, HSIL, or SCC.

Cervical DNA ploidy analysis

The specimens prepared through endocervical brushing were stained using a stoichiometric DNA staining method (Feulgen-Thionin) according to the method described by Feulgen. DNA image cytometry was performed using a DNA Ploidy Analysis System (Landing Medical Hi-Tech Co., Ltd., Shanghai, China) by measuring the nuclear characteristics and DNA distribution in all nucleated cells. The DNA ploidy result was expressed as a "c" value for chromosome. The DNA ploidy value of 2c was identical to that of normal diploid cells. Most studies have used 5c as the cut-off value to define aneuploidy. DNA ploidy positivity was defined as the presence of three or more cells with a DNA ploidy value greater than 5c.

Colposcope and pathological examination

Experienced gynecologists performed electronic colposcopy. The examinees were placed in the lithotomy position. After cervical exposure using a vaginal speculum, cervical secretions were removed using dry cotton balls for preliminary observation. Subsequently, 3% acetic acid was applied to the cervix and colposcopic images were carefully observed, recorded, and archived. In cases in which abnormalities were suspected, cervical biopsies were collected under colposcopic guidance and fixed in 1% formalin solution for subsequent histological examination.

Statistical analysis

Data were independently entered by two researchers (W.S. and H.L.) and cross-checked to avoid information bias. The sample size was calculated by using the equation of $N = Z_{\alpha/2} * P * (1 - P) / d^2$. $Z_{\alpha/2}$ indicates the Z score under the precise level of $\alpha/2$. P values indicate the sensitivity or specificity of the screening tools used in this study. d indicates margin of error. In this study, 0.05 was used for α and d. As the sensitivity and specificity of the screening tool may vary in different populations, 0.8 and 0.5 were used as P to obtain a range of target sample size, which were from 246 to 385. Continuous variables were presented as the mean [standard deviation (SD)], whereas count or categorical variables were presented as numbers (%). The crude and adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated using univariate and multivariate logistic regression models. Missing value was presented as "unknown" in *Table 1* and considered as one level for certain variable in the regression model. Age, number of induced abortions, menopausal status, and squamocolumnar junction (SCJ) visibility might be related to cervical malignant lesions and were adjusted for in the regression model. All data were analyzed using the R statistics package (R version 4.4.1; R: The R Project for Statistical Computing, Vienna, Austria).

Results

Overall description

Between November 1, 2021, and February 28, 2022, 388 women visited the Department of Gynecology at Ruijin

Table 1 Clinicopathologic characteristics of the study

Characteristics	Values (n=335)
Age (years)	
Mean (SD)	43.5 (12.3)
Median (Q1, Q3)	42.0 (34.0, 52.0)
Smoking, n (%)	
No	295 (88.1)
Yes	20 (6.0)
Unknown	20 (6.0)
Menopause, n (%)	
No	236 (70.4)
Yes	79 (23.6)
Unknow	20 (6.0)
No. of pregnancies, n (%)	
0	62 (18.5)
1–3	205 (61.2)
4 or more	48 (14.3)
Unknown	20 (6.0)
Mode of abortion (n=171), n (%)	
Spontaneous abortion	1 (0.6)
Ectopic pregnancy	1 (0.6)
Medical abortion	22 (12.8)
Induced abortion	154 (90.0)
No. of induced abortion (n=154), n (%)	
1–2	129 (83.8)
≥3	22 (14.3)
Unknown	3 (1.9)
No. of birth, n (%)	
0	90 (26.9)
1–2	218 (65.1)
≥3	7 (2.1)
Unknow	20 (6.0)
Mode of delivery (n=225), n (%)	
Vaginal delivery	154 (68.4)
Cesarean section	66 (29.3)
Vaginal delivery and cesarean section	5 (2.2)
History of LEEP, n (%)	
No	322 (96.1)
Yes	13 (3.9)
Type of HPV, n (%)	
16/18 of high risk	85 (25.4)
HPV 16	56 (16.7)
HPV 18	30 (9.0)
Other types of high risk	303 (90.4)
HPV 26	2 (0.6)
HPV 31	15 (4.5)
HPV 33	14 (4.2)
HPV 35	9 (2.7)
HPV 39	24 (7.2)
HPV 45	6 (1.8)
HPV 51	28 (8.4)
HPV 52	76 (22.7)
HPV 53	42 (12.5)
HPV 56	34 (10.1)
HPV 58	49 (14.6)
HPV 59	18 (5.4)
HPV 66	23 (6.9)
HPV 68	16 (4.8)
HPV 73	6 (1.8)
HPV 82	3 (0.9)
Unknown	2 (0.6)
DNA ploidy, n (%)	
Negative	269 (80.3)
Positive (≥1)	66 (19.7)
Cervical cytology using Thinprep (TCT), n (%)	
NILM	287 (85.7)
ASC-US	21 (6.3)
ASC-H	4 (1.2)
LSIL	22 (6.6)
HSIL	1 (0.3)

Table 1 (*continued*)

Characteristics	Values (n=335)
Transformation zone of SCJ, n (%)	
1	44 (13.1)
2	48 (14.3)
3	242 (72.2)
Unknown	1 (0.3)
Histological diagnosis of cervical biopsy, n (%)	
NILM	180 (53.7)
LSIL	107 (31.9)
HSIL	45 (13.4)
Cervical cancer	3 (0.9)

SD, standard deviation; Q1, the first quartile; Q3, the third quartile; SCJ, squamocolumnar junction; NILM, negative for intraepithelial lesions or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells-cannot exclude high-grade lesions; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions; HPV, human papillomavirus.

Hospital for screening. After excluding 16 women with non-standard TCT results, 3 with intrauterine adhesions, 19 with a history of cervical cancer, and 15 with incomplete colposcopy or screening test information, 335 women were included in the analysis. Their mean age was 43.5 years (SD 12.3). Of these patients, 48 (14.3%) were diagnosed with cervical cancer or HSIL on biopsy (*Table 1*). Among the participants, 20 (6%) had a history of smoking, 79 (23.6%) were postmenopausal, and the majority (205, 61.2%) had been pregnant 1–3 times. Miscarriages occurred in 171 (67.6%) of those who had been pregnant, predominantly through induced abortion (154, 90.0%). High-risk HPV 16/18 was found in 85 (25.4%) participants, whereas 303 (90.4%) tested positive for other high-risk HPV types with some coinfection. DNA ploidy testing revealed abnormalities in 66 patients (19.7%). The TCT results showed that 85.7% of the participants had NILM, 22 (6.5%) had LSIL, and only one (0.3%) had HSIL.

Classification model (Table 2)

HPV 16/18 infection, TCT, and DNA ploidy tests are critical for predicting cervical cancer and precancerous lesions. Individually, these predictors showed varying sensitivities and specificities (*Table 2* and *Figure 2*). HPV 16/18 and TCT demonstrated low sensitivities of 35.4% and 29.2% but relatively high specificities of 76.1% and 88.2%, respectively. The positive predictive values (PPVs) were modest, at 20.0% for HPV 16/18 and 29.2% for TCT, whereas the negative predictive values (NPVs) were close to 90%. Their classification accuracies were 70.3% and 79.7%, respectively, with Youden Indices ranging from 11% to 18%. In contrast, the DNA ploidy test exhibited high sensitivity and specificity, exceeding 90%, with PPV and NPV of 68.2% and 98.9%, respectively, resulting in a predictive accuracy of 92.8% and a Youden Index of 86.4%.

When combining HPV 16/18 and TCT, the sensitivity increased to 60.4%, but the specificity decreased to 65.7%. The overall accuracy of the combined model was only 65.0%, with a Youden index of 13.7%.

We also evaluated the performance of combinations involving single predictors, HPV 16/18 and TCT, using the better-performing DNA ploidy test (*Table 2*, *Figure 3*). The combined DNA ploidy and TCT model showed good sensitivity [93.8% (95% CI: 86.9–100%)] and specificity [82.6% (95% CI: 78.2–87.0%)] but did not perform better than DNA ploidy alone. Similarly, the DNA ploidy and HPV 16/18 models, as well as the tri-combination with all three predictors, yielded high sensitivity [97.9% (95% CI: 93.9–100%)] but low specificity, particularly in the three-way combination [62.2% (95% CI: 56.6–67.9%)] (*Table 2*, *Figure 4*). When applying DNA ploidy alone to classify cervical cancer or precancerous lesions in participants negative for HPV 16/18 and TCT (*Table 2*, *Figure 4*), both sensitivity and specificity reached 94.7%, with an accuracy and Youden index of 94.7% and 89.4%, respectively. This approach identified 18 additional cases (8.7% of 207) that were initially negative for HPV 16/18 and TCT and were subsequently confirmed by pathology.

Risk factors of cervical cancer or precancerous lesions

Univariate analysis was initially used to estimate the crude OR for key predictive factors among the 48 HSIL or cervical cancer cases. HPV 16/18 did not show a statistically significant association with increased risk (OR: 1.75; 95% CI: 0.90–3.32), unlike the other factors where DNA ploidy demonstrated a strong association (OR: 190.0; 95% CI: 62.9–830.6) and TCT showed moderate significance (OR: 3.06; 95% CI: 1.46–6.21). Multivariable logistic regression adjusted for age, number of induced abortions, menstrual status, and SCJ results indicated that the influence of

Table 2 Classification predictive model for single or combinational factors

Detection method	Histological diagnosis of HSIL or cervical cancer		Positive ratio	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy*	Youden index
	–	+							
HPV 16/18			25.5%	35.4% (21.9–49.0%)	76.1% (71.2–81.1%)	20.0% (13.1–26.9%)	87.5% (85.1–89.9%)	70.3%	11.6%
–	217	31							
+	68	17							
NA	2	0							
TCT			14.3%	29.2% (16.3–42.0%)	88.2% (84.4–91.9%)	29.2% (18.0–40.4%)	88.2% (86.2–90.1%)	79.7%	17.3%
–	253	34							
+	34	14							
DNA ploidy			19.7%	93.8% (86.9–100.0%)	92.7% (89.7–95.7%)	68.2% (59.1–77.3%)	98.9% (97.7–100.0%)	92.8%	86.4%
–	266	3							
+	21	45							
HPV 16/18 + TCT			37.9%	60.4% (46.6–74.3%)	65.7% (60.2–71.2%)	22.8% (17.9–27.8%)	90.8% (87.8–93.8%)	65.0%	13.7%
–	188	19							
+	98	29							
NA	1	0							
HPV 16/18 + DNA ploidy			39.0%	97.9% (93.9–100.0%)	70.9% (65.6–76.1%)	36.2% (31.9–40.4%)	99.5% (98.6–100.0%)	74.8%	97.4%
–	202	1							
+	83	47							
NA	2	0							
TCT + DNA ploidy			28.4%	93.8% (86.9–100.0%)	82.6% (78.2–87.0%)	47.4% (40.8–53.9%)	98.8% (97.4–100.0%)	84.2%	76.3%
–	237	3							
+	50	45							
TCT + HPV 16/18 + DNA ploidy			46.4%	97.9% (93.9–100.0%)	62.2% (56.6–67.9%)	30.3% (27.1–33.6%)	99.4% (98.4–100.0%)	67.4%	60.2%
–	178	1							
+	108	47							
NA	1	0							
DNA ploidy (patients with HPV 16/18– & TCT–)			13.5%	94.7% (84.7–100.0%)	94.7% (84.7–100.0%)	64.3% (50.2–78.3%)	99.4% (98.4–100.0%)	94.7%	89.4%
–	178	1							
+	10	18							

*, accuracy = (true positive + true negative)/(true positive + true negative + false positive + false negative). "+" indicated the positive result while "–" indicated the negative result of the corresponding test method. CI, confidence interval; HSIL, high-grade squamous intraepithelial lesions; HPV, human papillomavirus; TCT, ThinPrep cytology test.

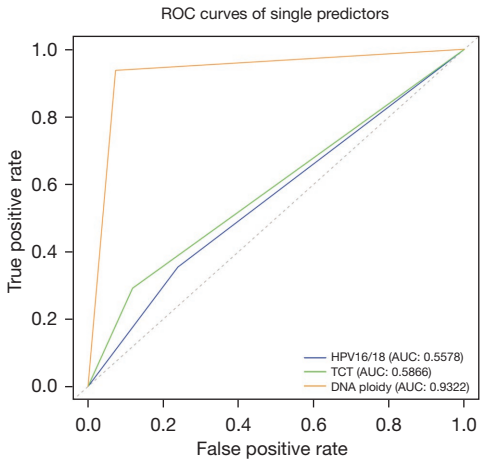

Figure 2 ROC curves of the single predictor. Blue line indicates participants with positive HPV subtype 16/18 detection. Green line indicates participants with positive TCT results (not NILM). Orange line indicates participants with DNA ploidy analysis over 5c. AUC, area under the curve; HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancy; ROC, receiver operating characteristic; TCT, ThinPrep cytology test.

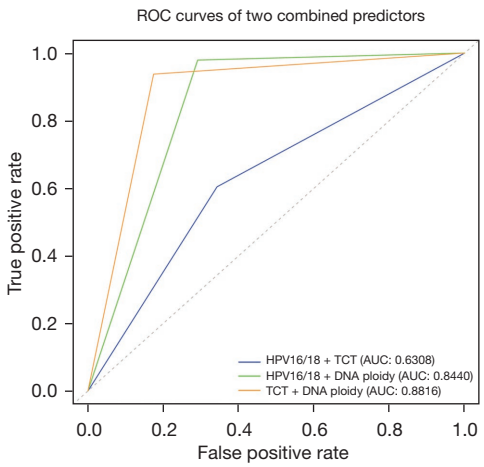

Figure 3 ROC curves of two combined predictor. Blue line indicates participants with either positive results in HPV subtype 16/18 detection or TCT testing. Green line indicates participants with either positive results in HPV subtype 16/18 detection or DNA ploidy analysis over 5c. Orange line indicates participants with positive results in TCT testing and DNA ploidy analysis over 5c. AUC, area under the curve; HPV, human papillomavirus; ROC, receiver operating characteristic; TCT, ThinPrep cytology test.

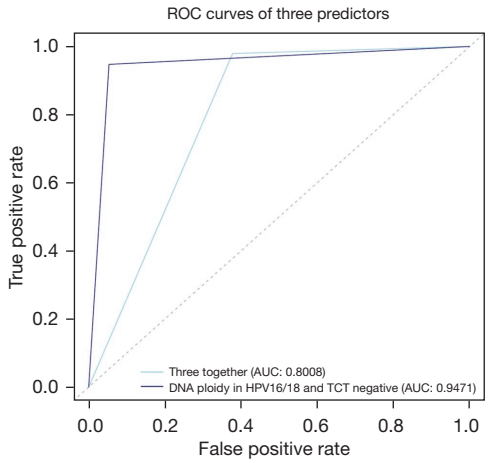

Figure 4 ROC curves of three combined predictor. Light blue line indicates the performance of predicting value if one of the three test obtained positive result. The dark blue line indicates DNA ploidy analysis performance when negative results shown in HPV 16/18 and TCT. AUC, area under the curve; HPV, human papillomavirus; ROC, receiver operating characteristic; TCT, ThinPrep cytology test.

DNA ploidy remained robust (OR: 290.40; 95% CI: 7.89–1913.70). However, the association between HPV 16/18 and TCT was not significant in this adjusted model (HPV: OR, 1.87; 95% CI: 0.59–6.15; TCT: OR, 1.65; 95% CI: 0.49–5.82) (*Table 3*).

Discussion

The World Health Organization (WHO) 2021 guidelines recommend HPV-DNA detection as the primary screening method for cervical cancer prevention (12). In this study, the detection rate for high-risk HPV 16/18 subtypes was 25.5%, which is significantly higher than the 5–15% typically reported for first-time screening (13). This discrepancy may be attributed to the nature of our study population, which primarily consisted of symptomatic individuals rather than healthy participants who are routinely screened based on age. In our study, the sensitivity and specificity of HPV-16/18 testing were 35.4% and 76.1%, respectively. These figures are lower than those reported in other studies but may more accurately reflect real-world performance (14-16). A notable strength of our study is the consistency in sample

Table 3 Crude and adjusted odds ratios for different risk factors

Variable	Crude OR (95% CI) in univariate regression	Crude OR (95% CI) in multivariate regression	Adjusted OR (95% CI)
HPV 16/18 (+ *vs.* –)	1.75 (0.90–3.32)	1.90 (0.65–5.84)	1.87 (0.59–6.15)
TCT (+ *vs.* –)	3.06 (1.46–6.21)	1.50 (0.48–4.81)	1.65 (0.49–5.82)
DNA ploidy (+ *vs.* –)	190.00 (62.90–830.60)	177.20 (57.90–783.20)	290.40 (7.87–1,913.70)
Age (per 10 years increase)	1.00 (0.97–1.03)	–	1.02 (0.52–1.99)
Number of induced abortions			
None	Ref		Ref
1–2 times	0.99 (0.91–1.07)		0.75 (0.21–2.55)
3 or more	1.14 (0.98–1.33)		9.59 (1.19–9.49)
Menopausal status			
Premenopausal	Ref		Ref
Postmenopausal	1.03 (0.94–1.13)		0.89 (0.15–5.31)
Unknown	1.01 (0.86–1.19)		0.24 (0.04–1.34)
SCJ visibility			
1	Ref		Ref
2	0.92 (0.80–1.06)		0.58 (0.08–4.02)
3	0.93 (0.83–1.04)		0.89 (0.04–1.34)

CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; SCJ, squamocolumnar junction; TCT, ThinPrep cytology test; Ref, reference.

collection, which was performed by the same clinician to minimize biases associated with sample preparation across the three tests.

Typically, individuals without HPV 16/18 infection but with a high risk of HPV infection, indicated as HPV subtypes 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82, are recommended for validation by cytological testing such as TCT. The specificity of the TCT results in our study was 88.2%, which is consistent with other studies, ranging from 86–100% (17). Owing to limited reproducibility and sensitivity, the false-NPV of the TCT is quite high, mostly over 50% (18-21). In addition, low levels of infection may result in false-negative results for HPV detection. Taken together, under current clinical practice, individuals with negative results for HPV 16/18 and TCT are recommended for subsequent follow-up. Therefore, the false-negative portion of individuals misses the optimal screening and treatment period.

In this study, we evaluated DNA ploidy as a triage test for women with positive HPV DNA test results and compared it with partial HPV 16/18 genotyping and TCT triage. DNA ploidy demonstrated a higher sensitivity and specificity for HSIL+ than those demonstrated by the combination of HPV 16/18 and TCT (93.8% *vs.* 60.4% and 92.7% *vs.* 65.7%, respectively). Additionally, its lower positivity rate (19.7% *vs.* 37.9%) suggests a reduced referral rate for colposcopy, which is highly advantageous in triage settings.

These findings may be linked to biological processes associated with HPV infection. Previous studies have indicated that abnormal DNA ploidy is observed in women with squamous intraepithelial lesions (SILs) infected with high-risk HPV types (22,23). The severity of SIL is positively correlated with the proportion of heteroploid cells. This correlation could stem from HPV DNA fragments integrating into the host's nuclear DNA within cervical epithelial cells, leading to aberrant cell division processes, such as multipolar mitosis and unbalanced chromosome division. These processes result in changes in chromosome number and structure, an increase in nuclear area, and alterations in DNA content and ploidy status. Therefore, DNA ploidy analysis may serve as an effective triage method for women with positive primary HPV test

results.

There are several limitations in this study. First, the sample size was modest, with only 335 women participating, including 43 who were diagnosed with HSIL or cervical cancer. Second, detecting post-infection DNA ploidy requires a certain period of persistent high-risk HPV infection before changes in host cells become detectable. All tests in this study were conducted within a brief 2-month period, thus the optimal timing for follow-up testing from HPV detection to DNA ploidy assessment remains unclear. Women with transient HPV infections may have biased the results. Owing to its cross-sectional retrospective design, future longitudinal multicenter studies are required to address this limitation. Third, the study participants were recruited from a single hospital, which may not adequately represent a broader population, thus limiting the generalizability of the findings. Finally, the limited number of covariates collected restricted our ability to explore potential subgroups that might respond differently to the screening tools. To address these limitations and validate the reliability of our findings, larger multicenter studies with long-term events are required.

Conclusions

This study demonstrated that DNA ploidy analysis offers superior specificity and sensitivity in the triage of women with a positive HPV test for detecting HSIL and cervical cancer, compared to triage using partial genotyping (HPV 16/18) and TCT. Notably, the DNA ploidy analysis revealed a lower positivity rate for triage than that of both HPV 16/18 and TCT. These findings suggest that DNA ploidy testing could serve as a more effective triage test than the currently recommended in the guidelines.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1455/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1455/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1455/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective study was exempt from review by the Ethics Committee of Ruijin Hospital because this type of study is a non-human subject research, and the need for informed consent was waived.

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