Clinical Epigenetics

RESEARCH

Open Access

Evaluating *PAX1/JAM3* methylation for triage in HPV 16/18-infected women

Jing Fei[1†], Lingyun Zhai[1†], Jing Wang[3†], Xiaoqing Zhu[1], Pei Liu[3], Linhai Wang[3], Dongxue Ma[3], Lei Li[2*] and Jianwei Zhou[1,4,5*]

Abstract

Objective Referring all women who tested positive for human papillomavirus (HPV) 16/18 to colposcopy may lead to potential over-referral issues. Triage tests based on cytology results face challenges in achieving accurate diagnoses. Our study aims to assess the clinical effectiveness of *PAX1/JAM3* methylation (CISCER) test as a triage method for HPV 16/18-positive women.

Methods From November 2021 to December 2022, a total of 334 women who tested positive for HPV 16/18 and were referred to colposcopy at The Second Affiliated Hospital of Zhejiang University School of Medicine were studied. The clinical utility of the CISCER test, cytology, and the combination of CISCER with cytology as potential triage tests was compared.

Results We observed a significant increase in the methylation levels of *PAX1* gene and *JAM3* gene in women with cervical intraepithelial neoplasia (CIN) grade 2 or severe (CIN2+). The CISCER test demonstrated superior triage performance over cytology, even when used in combination with cytology, showing a high sensitivity of 89.0% (95% confidence interval [CI] 82.9–95.1%) and specificity of 95.3% (95% CI 92.6–98.0%). It achieved an area under the curve of 0.921 (95% CI 0.877–0.966) and an odds ratio of 164.02 (95% CI 68.64–391.95). The immediate CIN2+ risk based on positive CISCER results would be 89.0% (95% CI 80.8–94.1%), with an estimated average of 1.12 referrals needed to detect one CIN2+ case. Moreover, CISCER triaging successfully identified all cancer patients and did not miss any CIN3+ cases among women aged ≥ 30.

Conclusions The *PAX1/JAM3* methylation detection exhibited excellent accuracy in identifying cervical precancerous lesions in HPV 16/18-positive women and could be considered as a triage tool to reduce excessive referrals for colposcopy and overtreatment.

Keywords *PAX1/JAM3* methylation, HPV 16/18-positive women, Triage performance, Colposcopy referrals

[†]Jing Fei, Lingyun Zhai and Jing Wang have contributed equally to this work.

*Correspondence:
Lei Li
lileigh@163.com
Jianwei Zhou
jianwei-zhou@zju.edu.cn
[1] Department of Gynecology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University, Hangzhou 310003, China
[2] Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China
[3] Department of Medical Laboratory, Beijing Origin-Poly Bio-Tec Co., Ltd., Beijing 102629, China
[4] Key Laboratory of Cancer Invasion and Metastasis (HUST), Ministry of Education, Wuhan 430000, China
[5] Zhejiang Provincial Clinical Research Center for Obstetrics and Gynecology, Hangzhou 310003, China

Introduction

Cervical cancer remains a significant global public health concern, with an estimated annual incidence of approximately 660,000 new cases and over 340,000 deaths worldwide [1]. The disease is mainly caused by persistent infection with high-risk human papillomavirus (hrHPV). The most cervical cancer-related human papillomavirus (HPV) types comprise a spectrum of high-risk oncogenic genotypes, encompassing up to 14 types. These include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, classified as Group 1 carcinogens by the International Agency for Research on Cancer, along with HPV66 and 68 [2]. Among these, HPV 16 and 18 pose the greatest threat, responsible for approximately 70% of all cervical cancer cases [3]. Early detection and accurate treatment strategies for hrHPV infection are crucial for the prevention of cervical cancer.

HPV nucleic acid testing is recommended as the primary screening method [4–6]. Women who test positive for HPV 16/18 are advised to undergo immediate referral for colposcopy in some countries, including China [7–10]. The World Health Organization (WHO) guidelines, specifically Algorithm 4, recommend that women who test positive for HPV16 or HPV18 should undergo visual inspection with acetic acid (VIA) to determine their eligibility for ablative treatment. This involves applying 3–5% acetic acid to the cervix, with or without magnification, to identify potential precancerous lesions [4]. The 2019 ASCCP Risk-Based Management Consensus Guidelines recommend immediate colposcopy for HPV16-positive women. For HPV18-positive women, the guidelines suggest co-testing (HPV and cytology) in 1 year [11]. However, HPV 16/18 infections do not invariably progress to severe lesions in the short term. Most HPV infections are transient, with the majority of women clearing the virus within 1–2 years post-exposure [12]. Referring women without high-grade lesions for colposcopy can result in unnecessary psychological distress, overtreatment, and inefficient utilization of medical resources [13]. The peak incidence of HPV16/18 infection typically occurs in women under 25 years old. However, most infections in this age group are transient, with approximately 70% clearing within 1–2 years. Consequently, immediate intervention for all HPV16/18-positive young women could potentially lead to overtreatment, posing unnecessary risks to future fertility [12, 14].

The latest WHO guidelines on screening and treatment of cervical precancerous lesions recommend considering emerging technologies, such as methylation tests, for triaging women after a positive HPV DNA test [4]. Gene methylation has been proven to be an effective molecular marker for early identification of individuals at high risk of cervical cancer [15–18]. Paired box gene1 (*PAX1*), an epithelial carcinoma-associated gene, has consistently shown value in detecting cervical cancer in numerous studies [19, 20]. Combining *PAX1* with the junctional adhesion molecule 3 (*JAM3*) gene in a methylation panel demonstrates outstanding performance in cervical screening [21–23]. In this article, we attempted to utilize *PAX1*/*JAM3* methylation for triaging HPV 16/18-positive women, to assess the impact of methylation detection on colposcopy referral guidance for this population.

Materials and methods

Study population

Patients who visited The Second Affiliated Hospital of Zhejiang University School of Medicine between November 2021 to December 2022 and tested positive for HPV 16/18 in outpatient opportunistic screening were enrolled in this study. The inclusion criteria included: (1) aged 18 years or older; (2) agreed to participate in the study and signed the informed consent form. The exclusion criteria were as follows: (1) history of HIV infection or immunodeficiency disease, or ongoing immunosuppressive drug treatment within the past 6 months; (2) history of reproductive tract-related tumors or organ transplantation; (3) currently pregnant or lactating; (4) vaginal douching, medication in the last 7 days, or sexual intercourse in the last 3 days. All participants were referred for colposcopy. Biopsies were conducted for abnormal lesions identified during colposcopy. For those with normal colposcopic findings, young women with normal/low-grade cytology results did not undergo biopsy following a comprehensive assessment of patient history, physical examination, and auxiliary tests; follow-up was recommended. In other cases, a random 1- to 2-point biopsy was performed with their consent. Biopsy specimens were evaluated by two experienced pathologists. Lesion staging included no cervical intraepithelial neoplasia (no-CIN), CIN1, CIN2, CIN3, and cervical cancer. The management of CIN and cancer cases followed the standard guidelines [5]. The final analysis excluded samples with insufficient DNA concentration in the cytology and methylation tests (Fig. 1).

This study strictly followed institutional and National Research Council ethics guidelines for research involving human subjects and was approved by the Human Research Ethics Committee of The Second Affiliated Hospital of Zhejiang University School of Medicine (IR2021001217).

Cytology tests

Cervical exfoliated cell specimens were collected using Rovers Cervex-Brush ((Rovers Medical Devices, Oss, The Netherlands) to collect cells from the cervix, which were then stored in of PreservCyt® liquid-based cytology

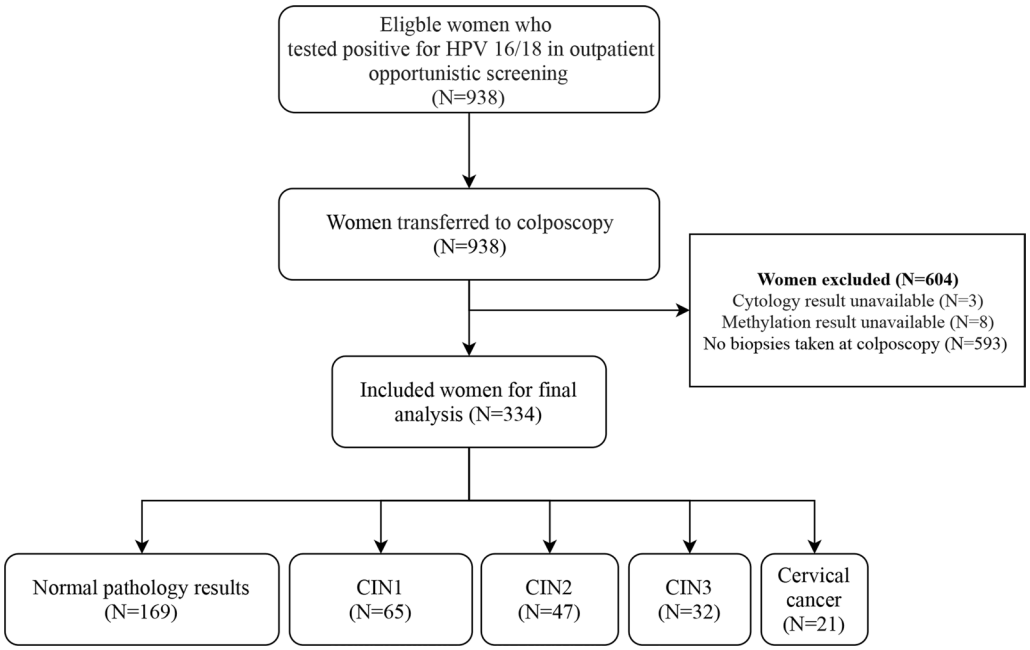

Fig. 1 Flowchart of recruitment for women tested positive for human papillomavirus (HPV) 16/18. *CIN* cervical intraepithelial neoplasia

medium (Hologic Inc, MA, USA). ThinPrep Cytologic Test (TCT) and imaging system were used for cytology testing [24]. Liquid-based cytology (LBC) results were classified according to the Bethesda 2014 classification criteria. The diagnoses in this study included: negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells which cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (SCC), and adenocarcinoma (AD).

High-risk HPV testing

The residual cervical specimens collected in the PreservCyt® medium were subsequently subjected to Cobas 4800 HPV testing (Roche Diagnostics, GmbH, Mannheim, Germany) following the manufacturer's instructions. The results of the Cobas 4800 test differentiate between HPV16/18 and other high-risk cancer-causing HPV genotypes (31/33/35/39/45/51/52/56/58/59/66/68).

PAX1/JAM3 methylation tests

The JH-DNA Isolation and Purifying kits (OriginPoly Bio-Tec Co., Ltd., Beijing, China) were used to extract Genomic DNA (gDNA) from the exfoliated cervical sample. Quantification of DNA concentration was performed using the NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, DE, USA). Bisulfite conversion of 200–1000 ng of gDNA was conducted with the JH-DNA Methylation-Lightning MagPrep (OriginPoly Bio-Tec Co., Ltd., Beijing, China). Following the manufacturer's instructions, the CISCER® DNA Methylation Detection Kit (OriginPoly Bio-Tec Co., Ltd., Beijing, China), which is approved by the China National Medical Products Administration (NMPA) as a Class III medical device (No. 20233400253), was utilized with the SLAN-96S real-time PCR System (Shanghai Hongshi Medical Technology Co., Ltd, China) to determine the methylation levels of *PAX1* (*PAX1*^{m}) and *JAM3* (*JAM3*^{m}). Using *GAPDH* as the internal control, the hypermethylation levels of the *PAX1* and *JAM3* genes were determined by calculating the difference between their respective Ct values ($\Delta CtP = Ct_{PAX1} - Ct_{GAPDH}$ and $\Delta CtJ = Ct_{JAM3} - Ct_{GAPDH}$). A positive result of the CISCER (*PAX1*^{m}/*JAM3*^{m}) test was defined as $\Delta CtP \leq 6.6$ or $\Delta CtJ \leq 10.0$. Methylation tests were conducted in a certified laboratory where technicians were blinded to the clinical information and other detection results of the patients.

Statistical analyses

Categorical variables were expressed as numbers with percentages, while continuous variables were presented as medians with interquartile ranges (Q1–Q3). Group comparisons for continuous variables utilized the Kruskal–Wallis rank sum test, whereas categorical variables were analyzed using Pearson's Chi-squared test or Fisher's exact test as appropriate. A significance threshold of $P < 0.05$ was applied. Receiver operating characteristic

curves (ROC), along with the corresponding areas under the curve (AUC) and their 95% confidence intervals, were generated using the pROC package (version 1.18.5). The report ROC (version 3.6) and epiR (version 2.0.75) packages were used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and odds ratio (OR), and the 95% confidence intervals, respectively. All statistical analyses were performed with R version 4.4.1.

Results

Characteristics of participants

According to the HPV genotyping results from outpatient opportunistic screening, a total of 938 women who tested positive for HPV 16 or 18 were enrolled in this study. All of these women were referred for colposcopy, and colposcopy-directed biopsies were performed in 345 cases. The remaining 593 women, who had normal colposcopic results, did not undergo biopsy due to their young age, normal or low-grade cytological findings, or refusal to consent to the procedure. Their clinical characteristics were detailed in Table S1. After excluding those with unavailable cytologist results ($n=3$) and invalid methylation tests ($n=8$), the final analysis included 334 women (Fig. 1). Among them, the majority ($n=245$, 73.4%) were infected with HPV 16, while 77 (23.0%) were infected with HPV 18. Additionally, twelve women (3.6%) tested positive for both HPV 16 and 18. The median age of the patients was 36 years (range 29–48), and there were no significant differences in age ($P=0.083$) observed among women with different HPV types (Table 1). Histopathological results showed that out of 334 cases, 169 (50.6%) were reported as normal, 65 (19.5%) were CIN1, 47 (14.1%) were CIN2, 32 (9.6%) were CIN3, and 21 (6.3%) were diagnosed with cervical cancer. Women infected with HPV 16 had a significantly higher prevalence of CIN2+ compared to those with HPV 18 (34.6% vs. 16.9%, $P=0.005$) (Table 1). However, Fisher's exact test indicated no statistically significant difference in cytological results between the two groups ($P=0.402$), with 57.6% of HPV 16-positive

Table 1 Characteristics of human papillomavirus (HPV) 16/18-positive women in this study

	Overall (*N*=334)	**HPV 16 (*N*=245)**	**HPV 18 (*N*=77)**	**HPV 16&18 (*N*=12)**	***P* value**
Age Median (Q1–Q3)	36 (29, 48)	37 (29, 49)	35 (27, 46)	32 (27, 36)	0.083
Pathology					0.005
Normal	169 (50.6%)	122 (49.8%)	39 (50.6%)	8 (66.7%)	
CIN1	65 (19.5%)	38 (15.5%)	25 (32.5%)	2 (16.7%)	
CIN2	47 (14.1%)	38 (15.5%)	8 (10.4%)	1 (8.3%)	
CIN3	32 (9.6%)	29 (11.8%)	2 (2.6%)	1 (8.3%)	
SCC	18 (5.4%)	16 (6.5%)	2 (2.6%)	0 (0%)	
AD	3 (0.9%)	2 (0.8%)	1 (1.3%)	0 (0%)	
Cytology					0.402
NILM	146 (43.7%)	104 (42.4%)	33 (42.9%)	9 (75.0%)	
ASC-US	94 (28.1%)	63 (25.7%)	29 (37.7%)	2 (16.7%)	
LSIL	56 (16.8%)	45 (18.4%)	10 (13.0%)	1 (8.3%)	
ASC-H	6 (1.8%)	5 (2.0%)	1 (1.3%)	0 (0%)	
HSIL	25 (7.5%)	22 (9.0%)	3 (3.9%)	0 (0%)	
Cervical cancer	7 (2.1%)	6 (2.4%)	1 (1.3%)	0 (0%)	
PAX1^{m}					< 0.001
Negative	251 (75.1%)	171 (69.8%)	70 (90.9%)	10 (83.3%)	
Positive	83 (25%)	74 (30%)	7 (9.1%)	2 (16.7%)	
JAM3^{m}					< 0.001
Negative	255 (76.3%)	177 (72.2%)	67 (87.0%)	11 (91.7%)	
Positive	79 (23.7%)	68 (27.8%)	10 (13.0%)	1 (8.3%)	
CISCER					< 0.001
Negative	234 (70.1%)	158 (64.5%)	66 (85.7%)	10 (83.3%)	
Positive	100 (29.9%)	87 (35.5%)	11 (14.3%)	2 (16.7%)	

CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; AD, adenocarcinoma; intraepithelial lesion or malignancy (NILM); ASC-US, atypical squamous cells of

women and 57.1% of HPV 18-positive women displaying cytological abnormalities. In terms of methylation testing, positivity rates for $PAX1^m$ (30.0% vs. 17.0%, $P < 0.001$), $JAM3^m$ (28.0% vs. 8.3%, $P < 0.001$), and their combination (CISCER: 35.5% vs. 14.3%, $P < 0.001$) were markedly higher in the HPV16-positive group than in the HPV18-positive group. This suggests a concordance between the results of methylation testing and histopathological findings.

PAX1/*JAM3* methylation in women with different grades of cervical lesions

The methylation results in women with different grades of cervical lesions are shown in Fig. 2. In the normal and CIN1 groups, 95.9% (162/169) and 93.9% (61/65) of women had no methylation in both *PAX1* and *JAM3* (Fig. 2a), respectively. However, the methylation levels of these two genes exhibited a significant increase from CIN1 to CIN2 stages, and escalated with the severity of the lesion (Fig. 2b, c). In women with CIN2, 78.7% (37/47) showed methylation in at least one gene, and

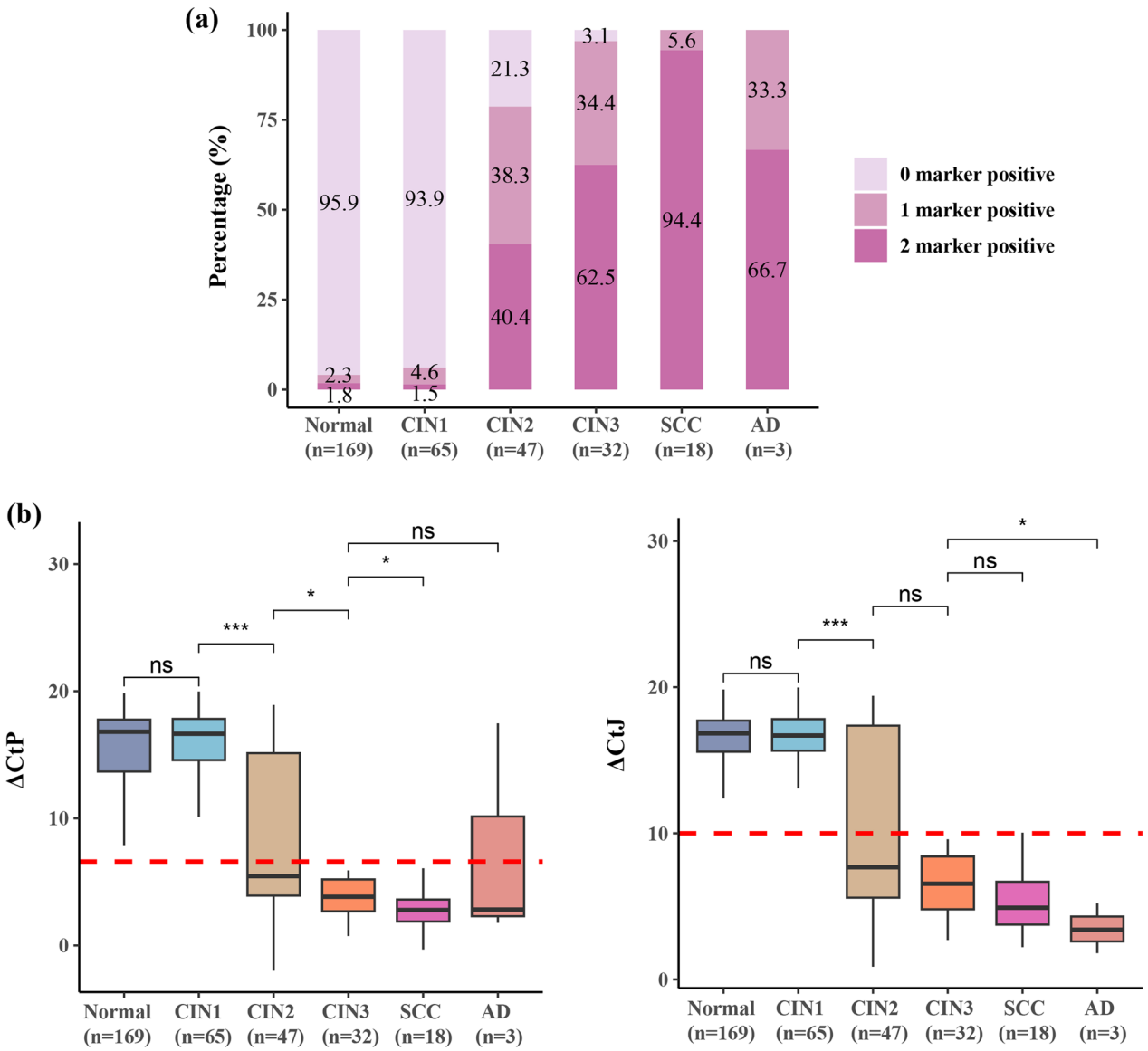

Fig. 2 The methylation of *PAX1* and *JAM3* gene in women with different grades of cervical lesions. **a** 0 marker positive: *PAX1* and *JAM3* methylation results were negative; 1 marker positive: *PAX1* or *JAM3* methylation results was positive; 2 marker positive: *PAX1* and *JAM3* methylation results were positive; **b**, **c** ΔCtP The ΔCt values of *PAX1* gene, ΔCtJ The ΔCt values of *JAM3* gene, CIN cervical intraepithelial neoplasia, SCC squamous cell carcinoma, AD adenocarcinoma

40.4% (19/47) exhibited methylation in both genes. In women with CIN3, these proportions increased to 96.9% (31/32) for at least one gene and 62.5% (20/32) for both genes positive, respectively. Furthermore, the methylation levels of *PAX1* and *JAM3* were slightly lower in younger women with advanced CIN2/3 lesions compared to older women, although these differences were not statistically significant (Figure S1).

Among the 18 women had squamous carcinoma and 3 women had AD, the positivity rate of CISCER methylation reached 100%. *PAX1* methylation was detected in all squamous carcinoma cases, while *JAM3* methylation was negative in one case (with cytological results indicating HSIL). In contrast, one woman with AD showed negative *PAX1* methylation (with LSIL cytological findings), whereas markedly high levels of *JAM3* methylation were observed in all AD cases. These findings underscore the complementary roles of *PAX1* and *JAM3* methylation, indicating a stronger association of *JAM3* with AD.

Clinical performance of *PAX1*/*JAM3* methylation for triage

We further analyzed the clinical efficacy of methylation and cytology tests for triaging HPV 16/18-positive women (Table 2). For detecting CIN2+, *PAX1* methylation showed a sensitivity of 75.0% (95% CI 66.5–83.5%) and a specificity of 96.6% (95% CI 94.3–98.9%). *JAM3* methylation demonstrated a sensitivity of 72.0% (95% CI 63.2–80.8%) and a specificity of 97.0% (95% CI 94.8–99.2%). Combining both biomarkers increased the sensitivity to 89.0% (95% CI 82.9–95.1%), while maintaining a high specificity of 95.3% (95% CI 92.6–98.0%). Such high specificity is particularly important in the triage of hrHPV-positive women.

When triaging with cytology ≥ ASC-US (including ASC-US, LSIL, ASC-H, HSIL, SCC and AD), its sensitivity (72.0%, 95% CI 63.2–80.8%) was inferior to CISCER test and exhibited lower specificity (50.4%, 95% CI 44.0–56.8%). For cytology ≥ HSIL, despite achieving higher specificity (97.9%, 95% CI 96.0–99.7%), sensitivity was notably low at 27.0% (95% CI 18.3–35.7%). Triaging with combination of CISCER and Cytology ≥ ASC-US achieved a higher sensitivity of 91.0% (95% CI 85.4–96.6%) for CIN2+, but its specificity dropped to 49.1% (95% CI 42.7–55.6%). In addition, the AUC (0.921: 95% CI 0.877–0.966) and odds ratio (164.02, 95% CI 68.64–391.95) of CISCER alone were higher compared to the combination of CISCER and cytology tests (≥ ASC-US or ≥ LSIL, ≥ HSIL).

Similar results were observed for CIN3+ outcomes, with CISCER test showing a sensitivity of 98.1% (95% CI 94.5–100%), specificity of 82.9% (95% CI 78.5–87.3%), an AUC of 0.905 (95% CI 0.865–0.945), and a odds ratio of

Table 2 Performance of methylation, cytology, and methylation/cytology to detect CIN2 + lesions among human papillomavirus (HPV) 16/18-positive women

	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	AUC (95% CI)	OR (95% CI)
CISCER	89.0 (82.9–95.1)	95.3 (92.6–98.0)	89.0 (82.9–95.1)	95.3 (92.6–98.0)	0.921 (0.877–0.966)	164.02 (68.64–391.95)
PAX1^{m}	75.0 (66.5–83.5)	96.6 (94.3–98.9)	90.4 (84.0–96.7)	90.0 (86.3–93.7)	0.858 (0.804–0.912)	84.75 (36.66–195.90)
JAM3^{m}	72.0 (63.2–80.8)	97.0 (94.8–99.2)	91.1 (84.9–97.4)	89.0 (85.2–92.9)	0.845 (0.790–0.900)	83.39 (34.95–198.96)
$Cytology_{ASC-US}$	72.0 (63.2–80.8)	50.4 (44.0–56.8)	38.3 (31.3–45.2)	80.8 (74.4–87.2)	0.612 (0.536–0.688)	2.62 (1.58–4.34)
$Cytology_{LSIL}$	50.0 (40.2–59.8)	81.2 (76.2–86.2)	53.2 (43.1–63.3)	79.2 (74.0–84.3)	0.656 (0.582–0.730)	4.32 (2.59–7.20)
$Cytology_{HISL}$	27.0 (18.3–35.7)	97.9 (96–99.7)	84.4 (71.8–97.0)	75.8 (71.0–80.7)	0.624 (0.572–0.677)	16.94 (6.29–45.59)
CISCER + $Cytology_{ASC-US}$	91.0 (85.4–96.6)	49.1 (42.7–55.6)	43.3 (36.6–50.0)	92.7 (88.2–97.3)	0.701 (0.641–0.761)	9.77 (4.70–20.3)
CISCER + $Cytology_{LSIL}$	89.0 (82.9–95.1)	78.2 (72.9–83.5)	63.6 (55.6–71.5)	94.3 (91.1–97.6)	0.836 (0.779–0.893)	29.03 (14.43–58.41)
CISCER + $Cytology_{HSIL}$	89.0 (82.9–95.1)	93.6 (90.5–96.7)	85.6 (78.8–92.3)	95.2 (92.5–98.0)	0.913 (0.867–0.959)	118.13 (52.23–267.16)

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; OR, odds ratio; CISCER, *PAX1*^{m}/*JAM3*^{m}; *PAX1*^{m}, the methylation of *PAX1* gene; *JAM3*^{m}, the methylation of *JAM3* gene; $Cytology_{ASC-US}$, the positive cytology results were defined as cytology ≥ ASC-US (ASC-US, LSIL, ASC-H, HSIL, SCC and AD); $Cytology_{LSIL}$, the positive cytology results were defined as cytology ≥ LSIL (LSIL, ASC-H, HSIL, SCC and AD); $Cytology_{HSIL}$, the positive cytology results were defined as cytology ≥ HSIL (HSIL, SCC and AD); CISCER + $Cytology_{ASC-US}$. The positive results were defined as positive CISCER results or cytology ≥ ASC-US; CISCER + $Cytology_{LSIL}$. The positive results were defined as positive CISCER results or cytology ≥ LSIL; CISCER + $Cytology_{HSIL}$. The positive results were defined as positive CISCER results or cytology ≥ HSIL

252.42 (95% CI 34.06–1870.65) (Table S2). These findings indicate that *PAX1*/*JAM3* methylation can effectively stratify low- and high-grade cervical lesions in HPV16/18-positive women, irrespective of cytology results.

Assessment of CIN2+ risk stratified by *PAX1*/*JAM3* methylation and potential impact on colposcopy referrals

Without triage, all HPV 16/18-positive women were referred to colposcopy. However, the immediate CIN2+ risk was 29.9%, requiring an average of 3.34 referrals to detect one CIN2+ case. Incorporating cytology ≥ ASC-US as a criterion for colposcopy referral would reduce this number to 2.61, but it would introduce a 28.0% (95% CI 19.7–38.0%) missed detection rate for CIN2+, including 14.3% (95% CI 3.8–37.4%) of women with cancer. Ignoring women with ASC-US could further increase the missed diagnosis rate to 50.0% (95% CI 40.4–59.6%).

By triaging with CISCER test, the estimated colposcopy referral rate would be reduced by nearly 70%, with an average of 1.12 referrals required to detect one CIN2+ case. The immediate CIN2+ risk based on positive CISCER results would be 89.0% (95% CI 80.8–94.1%), significantly higher than cytology (≥ ASC-US: 38.3% [95% CI 31.4–45.7%]; ≥ LSIL: 53.2% [95% CI 42.7–63.5%]), and even higher than the combining methylation and cytology (CISCER and Cytology ≥ ASC-US: 43.3% [95% CI 36.6–50.3%]; CISCER & Cytology ≥ LSIL: 63.6% [95% CI 55.0–71.4%]). Meanwhile, CISCER triaging accurately identified all cancer patients, 96.9% (31/32) of CIN3 patients and 78.8% (37/47) of CIN2 patients. When combining CISCER with cytology (≥ ASC-US), however, the number of referrals per CIN2+ detection would increase to 2.31 though the missed CIN2+ rate slightly decreased (11.0% [95% CI 5.9–19.2%]–9.0% [95% CI 4.5–16.8%]) (Table 3).

Among the 11 CIN2+ women who tested negative with CISCER test, 10 (90.9%) were under the age of 45. Analyzing results across different age groups, it was found that women aged < 30 has the lowest colposcopy referral rate (27.1%, 95% CI 18.8–37.3%) after CISCER triage, and the highest CIN2+ risk (96.2%, 95% CI 78.4–99.8%) with positive CISCER results. Moreover, no cancer cases were missed among these individuals, and 9 out of 10 CIN3 patients were successfully detected. On the other hand, women aged ≥ 45 exhibited the lowest rates of missed CIN2+ cases (2.9%), with the estimated unnecessary referral rate being less than 10%. Among women aged ≥ 30, CISCER triaging did not miss any CIN3+ patients. Overall, *PAX1*/*JAM3* methylation resulted in the lowest number of referrals needed to detect advanced lesions, while minimizing missed diagnoses in elder women.

Discussion

In our current cohort of 334 women positive for HPV 16/18, we assessed the clinical performance of *PAX1*/*JAM3* methylation across different grades of cervical lesions, and its effectiveness in triaging diagnosis. The methylation detection exhibited a good balance of sensitivity and specificity in CIN2 and CIN3 lesions, achieving AUC values of 0.921 and 0.905, respectively, outperforming cytology alone or cytology combined with methylation tests. Importantly, triaging using *PAX1*/*JAM3* methylation in HPV16/18-positive women can markedly reduce excessive colposcopy referrals without increasing the risk of missing high-grade lesions.

Given the high risk of HPV16/18 infections progressing to precancerous lesions, as evidenced by a 10-year follow-up study which found a progression rate of 20.7% for women infected with HPV16 and 17.7% for those infected with HPV18 [25], it is recommended that individuals who test positive for HPV 16 or 18 should be directly referred for colposcopy [7–10]. However, approximately 70% of HPV16/18-positive women in this study showed no lesions or only CIN1, consistent with findings from some larger cohort studies [26–28], suggesting that many women may not require immediate colposcopy referral. Using methylation tests to triage these populations can effectively reduce colposcopy referral rates. This approach is supported by previous reports on other gene methylation tests in hrHPV-positive women, such as WID-qCIN [29], *FAM19A4*/*miR124-2* [30], *ZNF671/ASTN1/ITGA4/RXFP3/SOX17/DLX1* [31], and *PAX1*/*SOX1* [32]. Particularly notable is the performance of *PAX1*/*JAM3* methylation in our study, demonstrating 89.0% sensitivity and 95.3% specificity in detecting CIN2+ lesions.

The clinical significance of the methylation of *PAX1* and *JAM3* in cervical cancer screening has been previously reported [19, 33–35]. *PAX1* gene expression can epigenetically activate a series of phosphatases, which subsequently suppress signaling pathways such as EGF/MAPK, thereby inhibiting malignant phenotypes. HPV infection can lead to high methylation of the *PAX1* gene, resulting in downregulation or loss of its expression [36, 37]. *JAM3* is involved in the formation of tight junctions between cells, facilitating the regulation of vascular permeability and the migration of leukocytes across endothelial surfaces [38]. Combining *PAX1* and *JAM3* into a methylation panel has demonstrated promising efficacy in studies involving patients with persistent HPV infection and self-sampling methods [21, 23, 39]. In this cohort, we highlight its triage value

Table 3 Estimation of colposcopy referrals for human papillomavirus (HPV) 16/18-positive women

	N (%)	CIN2+ Immediate risk % [95% CI]	CIN3+ Immediate risk % [95% CI]	Cervical cancer Immediate risk % [95% CI]	Estimated colposcopy referral rate	Colposcopy referrals required per CIN2+ detection	Missed CIN2+ % [95% CI]	Missed CIN3+ % [95% CI]	Missed cervical cancer %, [95% CI]
Total									
HPV16/18+	334	29.9 (100/334) [25.1–35.2]	15.9 (53/334) [12.2–20.3]	6.3 (21/334) [4.0–9.6]	100 (334/334) [98.6–100]	3.34 (334/100)	0 (0/100) [0–4.6]	0 (0/53) [0–8.4]	0 (0/21) [0–19.2]
Cytology ≥ ASC-US	188	38.3 (72/188) [31.4–45.7]	22.9 (43/188) [17.2–29.7]	9.6 (18/188) [5.9–14.9]	56.3 (188/334) [50.8–61.7]	2.61 (188/72)	28.0 (28/100) [19.7–38.0]	18.9 (10/53) [9.9–32.4]	14.3 (3/21) [3.8–37.4]
Cytology ≥ LSIL	94	53.2 (50/94) [42.7–63.5]	36.2 (34/94) [26.7–46.8]	16.0 (15/94) [9.5–25.3]	28.1 (94/334) [23.5–33.3]	1.88 (94/50)	50.0 (50/100) [40.4–59.6]	35.8 (19/53) [23.5–50.3]	28.6 (6/21) [12.2–52.3]
PAX1^{m}	83	90.4 (75/83) [81.4–95.4]	55.4 (46/83) [44.1–66.2]	24.1 (20/83) [15.7–35.0]	24.9 (83/334) [20.4–29.9]	1.11 (83/75)	25.0 (25/100) [17.1–34.8]	13.2 (7/53) [5.9–26]	4.8 (1/21) [0.2–25.9]
JAM3^{m}	79	91.1 (72/79) [82.0–96.1]	57.0 (45/79) [45.4–67.9]	25.3 (20/79) [16.5–36.6]	23.7 (79/334) [19.3–28.7]	1.1 (79/72)	28.0 (28/100) [19.7–38]	15.1 (8/53) [7.2–28.1]	4.8 (1/21) [0.2–25.9]
CISCER+	100	89.0 (89/100) [80.8–94.1]	52.0 (52/100) [41.8–62.0]	21 (21/100) [13.8–30.5]	29.9 (100/334) [25.1–35.2]	1.12 (100/89)	11.0 (11/100) [5.9–19.2]	1.9 (1/53) [0.1–11.4]	0 (0/21) [0–19.2]
CISCER+ or Cytology ≥ ASC-US	210	43.3 (91/210) [36.6–50.3]	24.8 (52/210) [19.2–31.3]	10.0 (21/210) [6.4–15.1]	62.9 (210/334) [57.4–68.0]	2.31 (210/91)	9.0 (9/100) [4.5–16.8]	1.9 (1/53) [0.1–11.4]	0 (0/21) [0–19.2]
CISCER+ or Cytology ≥ LSIL	140	63.6 (89/140) [55.0–71.4]	37.1 (52/140) [29.3–45.8]	15.0 (21/140) [9.7–22.2]	41.9 (140/334) [36.6–47.4]	1.57 (140/89)	11.0 (11/100) [5.9–19.2]	1.9 (1/53) [0.1–11.4]	0 (0/21) [0–19.2]
Age < 30									
HPV16/18+	96	30.2 (29/96) [21.5–40.6]	11.5 (11/96) [6.1–20.0]	1.0 (1/96) [0.1–6.5]	100 (96/96) [95.2–100]	3.31 (96/29)	0 (0/29) [0–14.6]	0 (0/11) [0–32.1]	0 (0/1) [0–94.5]
Cytology ≥ ASC-US	42	38.1 (16/42) [24.0–54.3]	16.7 (7/42) [7.5–32.0]	0 (0/42) [0–10.4]	43.8 (42/96) [33.8–54.2]	2.62 (42/16)	44.8 (13/29) [27.0–64.0]	36.4 (4/11) [12.4–68.4]	100 (1/1) [5.5–100]
Cytology ≥ LSIL	17	41.2 (7/17) [19.4–66.5]	23.5 (4/17) [7.8–50.2]	0 (0/17) [0–22.9]	17.7 (17/96) [10.9–27.1]	2.43 (17/7)	75.9 (22/29) [56.1–89.0]	63.6 (7/11) [31.6–87.6]	100 (1/1) [5.5–100]
PAX1^{m}	19	94.7 (18/19) [71.9–99.7]	42.1 (8/19) [21.1–66.0]	5.3 (1/19) [0.3–28.1]	19.9 (19/96) [12.6–29.4]	1.06 (19/18)	37.9 (11/29) [21.3–57.6]	27.3 (3/11) [7.3–60.7]	0 (0/1) [0–94.5]
JAM3^{m}	21	95.2 (20/21) [74.1–99.8]	33.3 (7/21) [15.5–56.9]	4.8 (1/21) [0.2–25.9]	21.9 (21/96) [14.3–31.7]	1.05 (21/20)	31 (9/29) [16.0–51.0]	36.4 (4/11) [12.4–68.4]	0 (0/1) [0–94.5]
CISCER+	26	96.2 (25/26) [78.4–99.8]	38.5 (10/26) [20.9–59.3]	3.8 (1/26) [0.2–21.6]	27.1 (26/96) [18.8–37.3]	1.04 (26/25)	13.8 (4/29) [4.5–32.6]	9.1 (1/11) [0.5–42.9]	0 (0/1) [0–94.5]
CISCER+ or Cytology ≥ ASC-US	52	48.1 (25/52) [34.2–62.2]	19.2 (10/52) [10.1–33.0]	1.9 (1/52) [0.1–11.6]	54.2 (52/96) [43.7–64.3]	2.08 (52/25)	13.8 (4/29) [4.5–32.6]	9.1 (1/11) [0.5–42.9]	0 (0/1) [0–94.5]
CISCER+ or Cytology ≥ LSIL	36	69.4 (25/36) [51.7–83.1]	27.8 (10/36) [14.8–45.4]	2.8 (1/36) [0.1–16.2]	37.5 (36/96) [28.0–48.0]	1.44 (36/25)	13.8 (4/29) [4.5–32.6]	9.1 (1/11) [0.5–42.9]	0 (0/1) [0–94.5]
Age > = 30 & < 45									
HPV16/18+	135	26.7 (36/135) [19.6–35.1]	15.6 (21/135) [10.1–23.0]	6.7 (9/135) [3.3–12.6]	100 (135/135) [96.6–100]	3.75 (135/36)	0 (0/36) [0–12.0]	0 (0/21) [0–19.2]	0 (0/9) [0–37.1]

Table 3 (continued)

	N (%)	CIN2+ Immediate risk % [95% CI]	CIN3+ Immediate risk % [95% CI]	Cervical cancer Immediate risk % [95% CI]	Estimated colposcopy referral rate	Colposcopy referrals required per CIN2+ detection	Missed CIN2+ % [95% CI]	Missed CIN3+ % [95% CI]	Missed cervical cancer %, [95% CI]
Cytology ≥ ASC-US	79	30.4 (24/79) [20.8–41.9]	21.5 (17/79) [13.4–32.5]	10.1 (8/79) [4.8–19.5]	58.5 (79/135) [49.7–66.8]	3.29 (79/24)	33.3 (12/36) [19.1–51.1]	19 (4/21) [6.3–42.6]	11.1 (1/9) [0.6–49.3]
Cytology ≥ LSIL	40	42.5 (17/40) [27.4–59.0]	32.5 (13/40) [19.1–49.2]	15 (6/40) [6.2–30.5]	29.6 (40/135) [22.2–38.2]	2.35 (40/17)	52.8 (19/36) [35.7–69.2]	38.1 (8/21) [19.0–61.3]	33.3 (3/9) [9.0–69.1]
PAX1^{m}	31	83.9 (26/31) [65.5–93.9]	58.1 (18/31) [39.3–74.9]	25.8 (8/31) [12.5–44.9]	23.0 (31/135) [16.4–31.1]	1.19 (31/26)	27.8 (10/36) [14.8–45.4]	14.3 (3/21) [3.8–37.4]	11.1 (1/9) [0.6–49.3]
JAM3^{m}	29	82.8 (24/29) [63.5–93.5]	65.5 (19/29) [45.7–81.4]	31.0 (9/29) [16.0–51.0]	21.5 (29/135) [15.1–29.5]	1.21 (29/24)	33.3 (12/36) [19.1–51.1]	9.5 (2/21) [1.7–31.8]	0 (0/9) [0–37.1]
CISCER +	37	81.1 (30/37) [64.3–91.4]	56.8 (21/37) [39.6–72.5]	24.3 (9/37) [12.4–41.6]	27.4 (39/135) [21.6–37.4]	1.23 (37/30)	16.7 (6/36) [7.0–33.5]	0 (0/21) [0–19.2]	0 (0/9) [0–37.1]
CISCER + or Cytology ≥ ASC-US	88	35.2 (31/88) [25.5–46.2]	23.9 (21/88) [15.7–34.4]	10.2 (9/88) [5.1–19.0]	65.2 (88/135) [56.5–73.0]	2.84 (88/31)	13.9 (5/36) [5.2–30.3]	0 (0/21) [0–19.2]	0 (0/9) [0–37.1]
CISCER + or Cytology ≥ LSIL	58	51.7 (30/58) [38.3–64.9]	36.2 (21/58) [24.3–49.9]	15.5 (9/58) [7.8–27.9]	43.0 (58/135) [34.6–51.8]	1.93 (58/30)	16.7 (6/36) [7.0–33.5]	0 (0/21) [0–19.2]	0 (0/9) [0–37.1]
Age > = 45									
HPV16/18 +	103	34.0 (35/103) [25.1–44.1]	20.4 (21/103) [13.3–29.7]	10.7 (11/103) [5.7–18.7]	100 (103/103) [95.5–100]	2.94 (103/35)	0 (0/35) [0–12.3]	0 (0/21) [0–19.2]	0 (0/11) [0–32.1]
Cytology ≥ ASC-US	67	47.8 (32/67) [35.6–60.2]	28.4 (19/67) [18.3–40.9]	14.9 (10/67) [7.8–26.2]	65.0 (67/103) [55.0–74.0]	2.09 (67/32)	8.6 (3/35) [2.2–24.2]	9.5 (2/21) [1.7–31.8]	9.1 (1/11) [0.5–42.9]
Cytology ≥ LSIL	37	70.3 (26/37) [52.8–83.6]	45.9 (17/37) [29.8–62.9]	24.3 (9/37) [12.4–41.6]	35.9 (37/103) [26.9–46.0]	1.42 (37/26)	25.7 (9/35) [13.1–43.6]	19.0 (4/21) [6.3–42.6]	18.2 (2/11) [3.2–52.2]
PAX1^{m}	33	93.9 (31/33) [78.4–98.9]	60.6 (20/33) [42.2–76.6]	33.3 (11/33) [18.6–51.9]	32.0 (33/103) [23.4–42.1]	1.06 (33/31)	11.4 (4/35) [3.7–27.7]	4.8 (1/21) [0.2–25.9]	0 (0/11) [0–32.1]
JAM3^{m}	29	96.6 (28/29) [80.4–99.8]	65.5 (19/29) [45.7–81.4]	34.5 (10/29) [18.6–54.3]	28.2 (29/103) [20.0–38.0]	1.04 (29/28)	20.0 (7/35) [9.1–37.5]	9.5 (2/21) [1.7–31.8]	9.1 (1/11) [0.5–42.9]
CISCER +	37	91.9 (34/37) [77.0–97.9]	56.8 (21/37) [39.6–72.5]	29.7 (11/37) [16.4–47.2]	35.9 (37/103) [26.9–46.0]	1.09 (37/34)	2.9 (1/35) [0.1–16.6]	0 (0/21) [0–19.2]	0 (0/11) [0–32.1]
CISCER + or Cytology ≥ ASC-US	70	50.0 (35/70) [38.6–61.4]	30.0 (21/70) [19.9–42.3]	15.7 (11/70) [8.5–26.8]	67.9 (70/103) [57.9–76.6]	2 (70/35)	0 (0/35) [0–12.3]	0 (0/21) [0–19.2]	0 (0/11) [0–32.1]
CISCER + or Cytology ≥ LSIL	46	73.9 (34/46) [58.6–85.2]	45.7 (21/46) [31.2–60.8]	23.9 (11/46) [13.1–39.1]	44.7 (46/103) [35.0–54.8]	1.35 (46/34)	2.9 (1/35) [0.1–16.6]	0 (0/21) [0–19.2]	0 (0/11) [0–32.1]

CIN, cervical intraepithelial neoplasia; CI, confidence interval; cytology ≥ ASC-US, cytology results of ASC-US, LSIL, ASC-H, HSIL, SCC and AD; cytology ≥ LSIL, cytology results of LSIL, ASC-H, HSIL, SCC and AD; *PAX1*^{m}, the methylation of *PAX1* gene; *JAM3*^{m}, the methylation of *JAM3* gene

in HPV 16/18-positive women. Assuming that a positive result would trigger a colposcopy referral, an average of only 1.12 referrals would be needed to detect one CIN2+ case. The findings of the study suggest that HPV16/18-positive women who test negative for *PAX1*^{m}/*JAM3*^{m} may not immediately require referral to colposcopy, especially young women.

As women age, there is a decline in hormone levels which leads to gradual changes in the cervix. This leads to the inward movement of the transformation zone into

the cervical canal and thinning of the cell layer, which poses challenges for the accuracy of cytology and colposcopy in detecting CIN2+lesions. Perimenopausal or postmenopausal women experience reduced natural clearance of HPV, making detection of HPV 16/18 infection in this age group of heightened clinical concern [40, 41]. Colposcopy poses challenges due to atrophy, retraction, and limited visualization of the transformation zone, potentially leading to misdiagnosis in cytology and biopsy [42]. Guidelines in some countries suggest considering diagnostic endocervical curettage (ECC) or large loop excision of the transformation zone (LLETZ) in women with abnormal screening results when visualization of the transformation zone is incomplete [43, 44]. LLETZ and excessive ECC not only increase the financial burden on patients, but also pose a higher risk of postoperative complications such as adhesions, pain, cervical stenosis, and others. Additionally, they decrease the likelihood of patients attending follow-up appointments. The CISCER triage strategy has demonstrated the ability to effectively identify high-risk patients without overtreating and to reduce missed diagnoses, particularly in women aged 45 and older. The promising efficiency of *PAX1*/*JAM3* methylation detection in our study suggests it could serve as a valuable risk marker to guide clinical management of older women, independent of cytological results.

Following CISCER triage, the colposcopy referral rate was lowest in the youngest age group, and the incidence of false-positive *PAX1*/*JAM3* methylation was lowest among these women. Regression rates for CIN2 are notably high in young women (< 30 years), reaching estimates of up to 70% at 3 years [45, 46]. Hence, women at this age are at higher risk of over-referral, which can be effectively minimized by methylation triage. A recent study demonstrated a marked decline in methylation positivity rates with decreasing age [47]. In our research, we observed lower *PAX1*/*JAM3* methylation levels in younger women. The difference in methylation levels by age may be correlate with spontaneous CIN2 regression rate and the duration of HPV infection [23]. Women with lower methylation levels may be less prone to progressing to higher-grade lesions [48]. For young women with higher HPV infection rates, CISCER triage indeed can alleviate potential anxiety and unnecessary surgical treatments. In China, where fertility rates have declined in recent years, more clinical data could be gathered on methylation testing as a triage method for HPV 16/18-positive young women with CIN2 lesions in the future.

All of the cervical cancer patients were identified through our methylation tests. The methylation of the *PAX1* and *JAM3* genes showed high consistency in risk stratification of women infected with HPV16/18. However, we observed that *PAX1*^{m} was negative in one adenocarcinoma patient, while the patient was not misdiagnosed due to positive result of *JAM3*^{m}. The incidence of cervical adenocarcinoma is lower than that of cervical squamous cell carcinoma, but its occurrence has been gradually rising in recent years. It usually originates within the cervical canal, characterized by multifocal or skip lesions. Conventional clinical examination methods have limited sensitivity, thereby increasing the risk of missed diagnoses and misdiagnosis [11, 49–51]. With advancements in molecular technology, an increasing number of markers and commercial tests are available for cancer detection. Nonetheless, it remains elusive whether certain markers are more closely associated with specific types of cancer. In future studies, we will expand our sample size of adenocarcinoma patients to more comprehensively evaluate the relationship between *JAM3* methylation and the development of adenocarcinoma.

In this study, *PAX1*^{m}/*JAM3*^{m} showed superior performance compared to cytology, and the combined effect of both was not significantly better than that of *PAX1*^{m}/*JAM3*^{m} alone. Higher levels of methylation directly correspond to more advanced cervical disease [48], while the cytology results rely on pathologists' interpretation [52]. The 8th National Congress of Colposcopy and Cervical Pathology (CSCCP) indicated that ASC-US/LSIL accounts for about 90% of cytological abnormalities in China. However, the incidence of high-grade lesions among these cases is generally below 30% [53–56], as observed in this study. Due to the low specificity of Cytology ≥ ASC-US/LSIL and the low sensitivity of Cytology ≥ HSIL, combining cytology with CISCER does not significantly enhance the triage performance.

With the WHO's strategy to eliminate cervical cancer introduced [57], prophylactic HPV vaccines have been recommended as a pivotal preventive measure. Their adoption has proven effective in reducing disease rates and the prevalence of HPV 16/18 [58, 59]. Despite this, HPV vaccine coverage remains notably low in China. The cumulative vaccination rate among women aged 9–45 was estimated to be less than 3% between 2018 and 2020 [60]. Therefore, cervical screening is still necessary. Early diagnosis and management strategies for women infected with hrHPV, especially HPV16/18, are currently focal points deserving significant attention. We propose that *PAX1*/*JAM3* methylation triage strategies can be instrumental in addressing these challenges.

There are also some limitations in this study. Histopathological results from biopsies, which may not always be conducted at the punctum maximum rather than through surgical procedures, could potentially introduce some bias into the outcomes of this study. Additionally, as a real-world study, not all referred colposcopy patients underwent

biopsies. This may cause a clinical selection/bias that could affect the performance of the methylation test and the accuracy of immediate CIN2/CIN3+ risk assessment. Future directions will involve expanding the sample size of patients with AD and conducting more rigorous clinical trials to evaluate the clinical value of methylation testing.

Conclusions

Overall, the *PAX1*/*JAM3* methylation test demonstrated high accuracy in identifying cervical CIN2 and severe lesions. Our data suggest that employing *PAX1*/*JAM3* methylation detection to further stratify HPV 16/18-positive women can decrease unnecessary referrals for colposcopy and the risk of overtreatment.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-024-01804-w.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
We express our gratitude for the support from the Open Project of Key Laboratory of Cancer Invasion and Metastasis (HUST), Ministry of Education.

Author contributions
JZ, LL and PL conceived and designed the work. JF, LZ and XZ collected the clinical data. LW and DM conducted the experiments. JW analyzed the data. JF, LZ and JW drafted the manuscript. JZ and LL revised the manuscript.

Funding
No fundings.

Availability of data and materials
The original contributions presented in the study were included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate
The study was approved by the Human Research Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine (IR2021001217).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 2 August 2024 Accepted: 16 December 2024
Published online: 26 December 2024

References

1. Bray F, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74:229–63. https://doi.org/10.3322/caac.21834.
2. Bouvard V, et al. A review of human carcinogens—part B: biological agents. Lancet Oncol. 2009;10:321–2. https://doi.org/10.1016/s1470-2045(09)70096-8.
3. Muñoz N, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348:518–27. https://doi.org/10.1056/NEJMoa021641.
4. Organization WH. 2021. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, second edition. Geneva: World Health Organization License: CC BY-NC-SA 3.0 IGO.
5. Bouvard V, et al. The IARC perspective on cervical cancer screening. N Engl J Med. 2021;385:1908–18. https://doi.org/10.1056/NEJMsr2030640.
6. Li MWL, Sui L, Ma D, Kong B, Wu X, Wu P, Qiao Y, Zhao F, Wang L. Guidelines for cervical cancer screening in China. Chin J Clin Obstet Gynecol. 2023;24:437–42. https://doi.org/10.13390/j.issn.1672-1861.2023.04.029.
7. Anonymous. Chinese expert consensus on the use of human papillomavirus nucleic acid testing for cervical cancer screening (2022). Zhonghua Yi Xue Za Zhi. 2023;103:1184–95. https://doi.org/10.3760/cma.j.cn112137-20230117-00096.
8. Massad LS, et al. Colposcopy standards: guidelines for endocervical curettage at colposcopy. J Low Genit Tract Dis. 2023;27:97–101. https://doi.org/10.1097/lgt.0000000000000710.
9. Chen FYZ, Sui L, Li S, Liu J, Liu A, Zhang W, Bi H, Geng L, Zhao Y, Wu D, Li F, Chen L, Duan X, Zhang S, Zhang G, Lang J. Chinese expert consensus on colposcopy application. Chin J Obstet Gynecol. 2020;55:443–9. https://doi.org/10.3760/cma.j.cn112141-20200320-00240.
10. Anonymous. 2022. Guidelines for Cervical Cancer Diagnosis and Treatment Available from http://www.nhcgovcn/yzygj/s2911/202204/a0e67177df1f439898683e1333957c74s.html
11. Perkins RB, et al. 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2020;24:102–31. https://doi.org/10.1097/lgt.0000000000000525.
12. Rodríguez AC, et al. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008;100:513–7. https://doi.org/10.1093/jnci/djn044.
13. Kyrgiou M, et al. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease. Cochrane Database Syst Rev. 2017;11:Cd012847. https://doi.org/10.1002/14651858.Cd012847.
14. Arbyn M, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. BMJ. 2008;337:a1284. https://doi.org/10.1136/bmj.a1284.
15. Steenbergen RD, et al. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14:395–405. https://doi.org/10.1038/nrc3728.
16. Johannsen E, Lambert PF. Epigenetics of human papillomaviruses. Virology. 2013;445:205–12. https://doi.org/10.1016/j.virol.2013.07.016.
17. Kremer WW, et al. The use of host cell DNA methylation analysis in the detection and management of women with advanced cervical intraepithelial neoplasia: a review. BJOG. 2021;128:504–14. https://doi.org/10.1111/1471-0528.16395.
18. Davalos V, Esteller M. Cancer epigenetics in clinical practice. CA Cancer J Clin. 2023;73:376–424. https://doi.org/10.3322/caac.21765.
19. Lai HC, et al. Identification of novel DNA methylation markers in cervical cancer. Int J Cancer. 2008;123:161–7. https://doi.org/10.1002/ijc.23519.
20. Xu J, et al. Assessing methylation status of PAX1 in cervical scrapings, as a novel diagnostic and predictive biomarker, was closely related to screen cervical cancer. Int J Clin Exp Pathol. 2015;8:1674–81.
21. Shang X, et al. A multicenter study on the accuracy of PAX1/JAM3 dual genes methylation testing for screening cervical cancer. Zhonghua Yi Xue Za Zhi. 2024;104:1852–9. https://doi.org/10.3760/cma.j.cn12137-20231004-00630.
22. Li X, et al. High-grade cervical lesions diagnosed by JAM3/PAX1 methylation in high-risk human papillomavirus-infected patients. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2023;48:1820–9. https://doi.org/10.11817/j.issn.1672-7347.2023.230175.
23. Li M, et al. PAX1/JAM3 methylation and HPV viral load in women with persistent HPV infection. Cancers (Basel). 2024. https://doi.org/10.3390/cancers16071430.
24. Makde MM, Sathawane P. Liquid-based cytology: technical aspects. Cytojournal. 2022;19:41. https://doi.org/10.25259/cmas_03_16_2021.

25. Khan MJ, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst. 2005;97:1072–9. https://doi.org/10.1093/jnci/dji187.
26. Gustinucci D, et al. Accuracy of different triage strategies for human papillomavirus positivity in an Italian screening population. Int J Cancer. 2022;150:952–60. https://doi.org/10.1002/ijc.33858.
27. Gu YY, et al. Evaluation of a methylation classifier for predicting precancer lesion among women with abnormal results between HPV16/18 and cytology. Clin Epigenet. 2020;12:57. https://doi.org/10.1186/s13148-020-00849-x.
28. Song F, et al. Triaging HPV-positive, cytology-negative cervical cancer screening results with extended HPV genotyping and p16(INK4a) immunostaining in China. BMC Infect Dis. 2021;21:400. https://doi.org/10.1186/s12879-021-06109-4.
29. Schreiberhuber L, et al. Cervical cancer screening using DNA methylation triage in a real-world population. Nat Med. 2024. https://doi.org/10.1038/s41591-024-03014-6.10.1038/s41591-024-03014-6.
30. Dick S, et al. Risk-stratification of HPV-positive women with low-grade cytology by FAM19A4/miR124-2 methylation and HPV genotyping. Br J Cancer. 2022;126:259–64. https://doi.org/10.1038/s41416-021-01614-4.
31. Zhang L, et al. Triage performance and predictive value of the human gene methylation panel among women positive on self-collected HPV test: results from a prospective cohort study. Int J Cancer. 2022;151:878–87. https://doi.org/10.1002/ijc.34041.
32. Fan C, et al. Analysis of the diagnostic performance of PAX1/SOX1 gene methylation in cervical precancerous lesions and its role in triage diagnosis. J Med Virol. 2024;96:e29521. https://doi.org/10.1002/jmv.29521.
33. Eijsink JJ, et al. A four-gene methylation marker panel as triage test in high-risk human papillomavirus positive patients. Int J Cancer. 2012;130:1861–9. https://doi.org/10.1002/ijc.26326.
34. Huang TH, et al. Quantitative analysis of methylation status of the PAX1 gene for detection of cervical cancer. Int J Gynecol Cancer. 2010;20:513–9. https://doi.org/10.1111/IGC.0b013e3181c7fe6e.
35. Yin A, et al. JAM3 methylation status as a biomarker for diagnosis of preneoplastic and neoplastic lesions of the cervix. Oncotarget. 2015;6:44373–87. https://doi.org/10.18632/oncotarget.6250.
36. Su PH, et al. Paired Box-1 (PAX1) activates multiple phosphatases and inhibits kinase cascades in cervical cancer. Sci Rep. 2019;9:9195. https://doi.org/10.1038/s41598-019-45477-5.
37. Wu W, et al. An overview of PAX1: expression, function and regulation in development and diseases. Front Cell Dev Biol. 2022;10:1051102. https://doi.org/10.3389/fcell.2022.1051102.
38. Weber C, et al. The role of junctional adhesion molecules in vascular inflammation. Nat Rev Immunol. 2007;7:467–77. https://doi.org/10.1038/nri2096.
39. Yu FM, Zhou J, Li X, G., et al. Feasibility evaluation of using PAX1/JAM3 methylation markers as cervical cancer screening for female self-collected samples. Chin J Lab Med. 2024;47:419–27. https://doi.org/10.3760/cma.j.cn114452-20240109-00016.
40. Smith JS, et al. Age-specific prevalence of infection with human papillomavirus in females: a global review. J Adolesc Health. 2008;43:S5-25. https://doi.org/10.1016/j.jadohealth.2008.07.009.
41. Li W, et al. Association of age and viral factors with high-risk HPV persistence: a retrospective follow-up study. Gynecol Oncol. 2019;154:345–53. https://doi.org/10.1016/j.ygyno.2019.05.026.
42. Fan A, et al. Analysis of clinical factors correlated with the accuracy of colposcopically directed biopsy. Arch Gynecol Obstet. 2017;296:965–72. https://doi.org/10.1007/s00404-017-4500-z.
43. Party CCACCSGW. 2020. Guidelines for the management of screen-detected abnormalities, screening in specific populations and investigation of abnormal vaginal bleeding. National Cervical Screening Program Sydney: Cancer Council Australia Available from: https://www.wikicancer orgau/australia/Guidelines:Cervical_cancer/Screening
44. Gustafson LW, et al. Clinical utility of p16/Ki67 dual-stain cytology for detection of cervical intraepithelial neoplasia grade two or worse in women with a transformation zone type 3: a cross-sectional study. BJOG. 2023;130:202–9. https://doi.org/10.1111/1471-0528.17248.
45. Tainio K, et al. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta-analysis. BMJ. 2018;360:k499. https://doi.org/10.1136/bmj.k499.
46. Moscicki AB, et al. Rate of and risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women. Obstet Gynecol. 2010;116:1373–80. https://doi.org/10.1097/AOG.0b013e3181fe777f.
47. Vink FJ, et al. FAM19A4/miR124-2 methylation testing and human papillomavirus (HPV) 16/18 genotyping in HPV-positive women under the age of 30 years. Clin Infect Dis. 2023;76:e827–34. https://doi.org/10.1093/cid/ciac433.
48. Kelly H, et al. Performance of DNA methylation assays for detection of high-grade cervical intraepithelial neoplasia (CIN2+): a systematic review and meta-analysis. Br J Cancer. 2019;121:954–65. https://doi.org/10.1038/s41416-019-0593-4.
49. Cleveland AA, et al. Cervical adenocarcinoma in situ: human papillomavirus types and incidence trends in five states, 2008–2015. Int J Cancer. 2020;146:810–8. https://doi.org/10.1002/ijc.32340.
50. Teoh D, et al. Diagnosis and management of adenocarcinoma in situ: a society of gynecologic oncology evidence-based review and recommendations. Obstet Gynecol. 2020;135:869–78. https://doi.org/10.1097/aog.0000000000003761.
51. Nikolopoulos M, et al. Cervical glandular neoplasia referrals and the diagnosis of adenocarcinoma in situ: correlating cytology, colposcopy findings, and clinical outcomes. Cytopathology. 2021;32:751–7. https://doi.org/10.1111/cyt.13027.
52. Sørbye SW, et al. Accuracy of cervical cytology: comparison of diagnoses of 100 Pap smears read by four pathologists at three hospitals in Norway. BMC Clin Pathol. 2017;17:18. https://doi.org/10.1186/s12907-017-0058-8.
53. Abdulaziz AMA, et al. Clinicopathologic significance and treatment of ASC-US in cervical cytology. Int J Clin Exp Pathol. 2020;13:307–16.
54. Xue H, et al. Use of extended HR-HPV Genotyping in improving the Triage Strategy of 2019 ASCCP recommendations in Women with positive HR-HPV diagnosis and simultaneous LSIL cytology results. J Cancer. 2021;12:4332–40. https://doi.org/10.7150/jca.55826.
55. Wang Y, et al. The efficiency of type-specific high-risk human papillomavirus models in the triage of women with atypical squamous cells of undetermined significance. Cancer Manag Res. 2020;12:5265–75. https://doi.org/10.2147/cmar.S254330.
56. Tao X, et al. Follow-up with histopathology and HPV testing on LSIL cytology in China's largest academic woman's hospital. Cancer Cytopathol. 2019;127:258–66. https://doi.org/10.1002/cncy.22119.
57. Organization WH. 2020. Global strategy to accelerate the elimination of cervical cancer as a public health problem.
58. Qiao YL, et al. Efficacy, safety, and immunogenicity of an *Escherichia coli*-produced bivalent human papillomavirus vaccine: an interim analysis of a randomized clinical trial. J Natl Cancer Inst. 2020;112:145–53. https://doi.org/10.1093/jnci/djz074.
59. Chu K, et al. Safety and immunogenicity of an Escherichia coli-produced 9-valent human papillomavirus L1 virus-like particle vaccine (types 6/11/16/18/31/33/45/52/58) in healthy adults: an open-label, dose-escalation phase 1 clinical trial. Lancet Reg Health West Pac. 2023;34:100731. https://doi.org/10.1016/j.lanwpc.2023.100731.
60. Song YLX, Yin Z, Yu W, Cao L, Cao L, Ye J, Wu J. Human papillomavirus vaccine coverage among the 9–45-year-old female population of China in 2018–2020. Chin J Vacc Immun. 2021;27:570–5. https://doi.org/10.19914/j.CJVI.2021101.

Publisher's Note