World Congress of the International Federation for Cancer Prevention and Colposcopy

19th Congress of IFCPC
Paris 2026

4 › 6 June 2026
Versailles - France
Palais des Congrès

Working to eliminate lower genital tract cancers

A six-methylation-marker panel as an effective triage alternative for HPV-positive women: a population-based prospective cohort

Liu Yang, Xiao Li
Zhejiang Provincial Clinical Research Center for Gynecological Diseases, Hangzhou 310006, China
Corresponding author: Xiao Li, Email: 5198008@zju.edu.cn

INTRODUCTION

Persistent high-risk HPV infection is the primary cause of cervical cancer, and while WHO recommends HPV DNA as the primary screening method, accurately triaging HPV-positive women to distinguish transient infections from progressive precancerous lesions (CIN2+/3+) remains a critical clinical need. DNA methylation markers, particularly the six-marker panel, have shown promising performance for risk stratification of CIN2+/CIN3+ in HPV-positive women and support extended retest intervals for triage-negative individuals.

OBJECTIVE

This study aimed to assess the triage performance of a six-gene methylation panel (ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671) in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and grade 3 or worse (CIN3+).

METHODS

We studied 603 HPV-positive women to evaluate a six-marker methylation panel for CIN2+/3+ risk stratification, comparing its triage performance with cytology using AUC, sensitivity, specificity, PPV, NPV, NRI and IDI.

RESULTS

Among the 603 women with high-risk HPV infection, 42/66 (6.97%/10.95%) women were diagnosed with CIN3+ at baseline and during the 3-year follow-up. The six-methylation panel showed a low positive rate in ≤CIN1 but a marked increase in CIN3+ lesions, with rates of 85.7% at baseline and 86.4% over 3-year follow-up (trend P <0.001). Combined with HPV16/18 genotyping, the methylation panel showed comparable baseline CIN3+ detection performance to cytology (all *P*>0.05) but significantly superior 3-year cumulative performance (AUC: 0.81 vs. 0.69, sensitivity: 90.9% vs. 66.7%, NPV: 98.5% vs. 94.5%, all *P*<0.05), with significant improvements in risk classification confirmed by categorical NRI (0.25, *P*<0.001) and IDI (0.11, *P*<0.001).

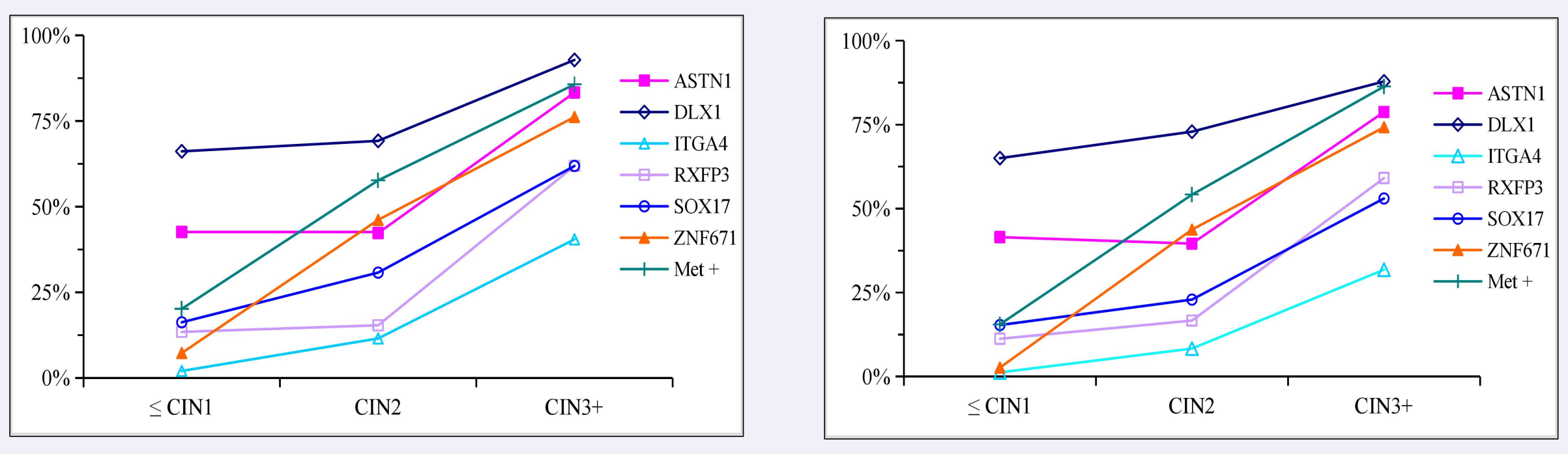

Figure1. Methylation positivity by cervical lesion: baseline (left) and 3-year follow-up (right)

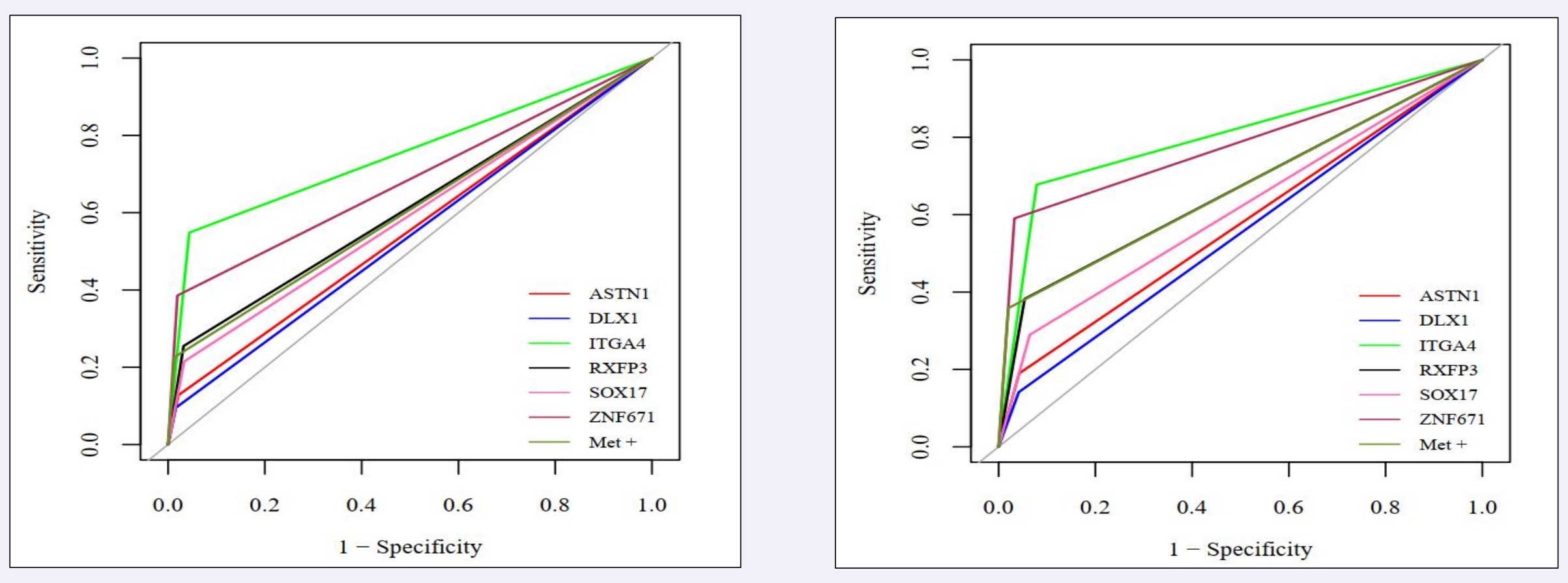

Figure 2. ROC curves for CIN3+ detection: baseline (left) and 3-year follow-up (right)

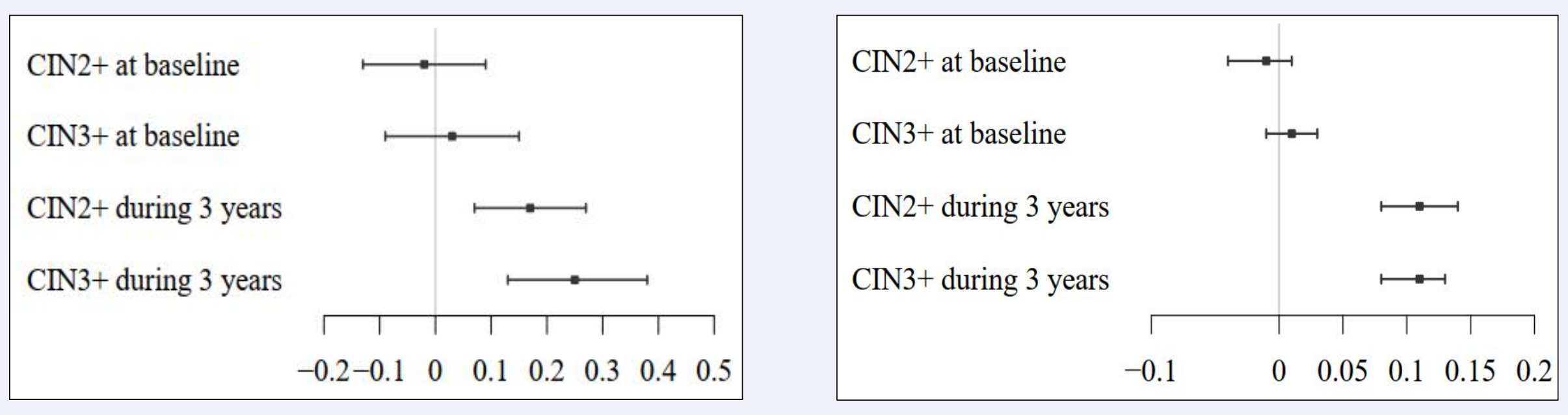

Figure 3. NRI (left) and IDI (right) comparison for CIN3+ detection in HPV-positive women

Table 1. Performance of cytologic, methylation, HPV16/18, and combinations in CIN3+ detection

	Cytologic findings		**Methylation testing**			**HPV16/18 and cytology**		**HPV16/18 and Met**		
Characteristic	**No./Total No.**	**% (95% CI)**	**No./Total No.**	**% (95% CI)**	***P***	**No./Total No.**	**% (95% CI)**	**No./Total No.**	**% (95% CI)**	***P***
Threshold	ASC-US+		Met positive			Either ASC-US+ or HPV16/18+		Either methylation positive or HPV16/18+		
Positivity	136/603	22.6 (19.3-26.1)	159/603	26.4 (22.9-30.1)	0.141	200/603	33.2 (29.4-37.1)	211/603	35.0 (31.2-39.0)	0.543
Detection of CIN3+ at baseline (n=42)										
AUC		0.79 (0.72-0.86)		0.82 (0.76-0.88)	0.430		0.78 (0.73-0.84)		0.80 (0.75-0.85)	0.609
Sensitivity	32/42	76.2 (60.2-87.4)	36/42	85.7 (70.8-94.1)	0.405	36/42	85.7 (70.8-94.1)	38/42	90.5 (76.5-96.9)	0.736
Specificity	457/561	81.5 (77.9-84.5)	438/561	78.1 (74.4-81.4)	0.181	397/561	70.8 (66.8-74.5)	388/561	69.2 (65.1-72.9)	0.602
PPV	32/136	23.5 (16.9-31.7)	36/159	22.6 (16.6-30.1)	0.967	36/200	18.0 (13.1-24.2)	38/211	18.0 (13.2-24.0)	1.000
NPV	457/467	97.9 (96.0-98.9)	438/444	98.6 (96.9-99.4)	0.512	397/403	98.5 (96.6-99.4)	388/392	99.0 (97.2-99.7)	0.784
Detection of CIN3+ during 3 years (n=66)										
AUC		0.68 (0.62-0.74)	0.84 (0.79-0.88)		<0.001		0.69 (0.63-0.75)		0.81 (0.77-0.85)	<0.001
Sensitivity	36/66	54.5 (41.9-66.7)	57/66	86.4 (75.2-93.2)	<0.001	44/66	66.7 (53.9-77.5)	60/66	90.9 (80.6-96.3)	0.001
Specificity	437/537	81.4 (77.8-84.5)	435/537	81.0 (77.4-84.2)	0.938	381/537	70.9 (66.9-74.7)	386/537	71.9 (67.8-75.6)	0.787
PPV	36/136	26.5 (19.5-34.8)	57/159	35.8 (28.5-43.9)	0.109	44/200	22.0 (16.6-28.5)	60/211	28.4 (22.6-35.1)	0.166
NPV	437/467	93.6 (90.9-95.6)	435/444	98.0 (96.0-99.0)	0.002	381/403	94.5 (91.7-96.5)	386/392	98.5 (96.5-99.4)	0.005

ASC-US, atypical squamous cells of undetermined significance; AUC, area under the curve; CI, confidence interval; CIN, cervical intraepithelial neoplasia (grades 1-3); HPV, human papillomavirus; HPV16/18+, positive for either HPV16 or HPV18; NPV, negative predictive value; PPV, positive predictive value.

CONCLUSION

The methylation panel exhibits robust screening efficacy for CIN2+/CIN3+ in HPV-positive women, with particular advantages in long-term risk prediction. It is a promising tool for refining risk stratification and strengthening cervical cancer prevention efforts.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209-49.
2. Chan KKL, Liu SS, Wei N, Ngu SF, Chu MMY, Tse KY, et al. Primary HPV testing with cytology versus cytology alone in cervical screening-A prospective randomized controlled trial with two rounds of screening in a Chinese population. Int J Cancer. 2020;147:1152-62.
3. Adcock R, Nedjai B, Lorincz AT, Scibior-Bentkowska D, Banwait R, Torrez-Martinez N, et al. DNA methylation testing with S5 for triage of high-risk HPV positive women. Int J Cancer. 2022;151:993-1004.
4. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, 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.