

HPV E6/E7 mRNA versus HPV DNA co-testing and colposcopy referral in adolescent and young adult women: a real-world exploratory triage study

Shuailiang Xin

The Third Affiliated Hospital of Zhengzhou University

tong xue

The Third Affiliated Hospital of Zhengzhou University

yunling li

The Third Affiliated Hospital of Zhengzhou University

mengke qin

The Third Affiliated Hospital of Zhengzhou University

yixuan liu

The Third Affiliated Hospital of Zhengzhou University

Yongzhen Guo

The Third Affiliated Hospital of Zhengzhou University

Wei Zhang

13803897097@163.com

The Third Affiliated Hospital of Zhengzhou University

Research Article

Keywords: Cervical cancer screening, Human papillomavirus, Triage, Adolescent and young adult, Verification bias

Posted Date: September 29th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10829598/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

In adolescent and young adult (AYA) women, high-risk human papillomavirus (HR-HPV) infection is often transient, so direct colposcopy referral of all HR-HPV-positive women may create avoidable burden. In Chinese hospital-based screening, HPV DNA genotyping and HPV E6/E7 mRNA assays coexist, but AYA-specific triage evidence is limited. We evaluated age-specific screening findings and modelled referral-detection trade-offs, treating platform comparisons as ecological.

Methods

We analysed women undergoing opportunistic cervical screening at a tertiary hospital in Henan, China, in 2023–2024. Women were classified as AYA (15–39 years) or non-AYA (≥ 40 years); 32,737 with valid cytology formed the analytic cohort. HR-HPV testing used either the Kaineng 23-genotype DNA assay or the Hologic Aptima E6/E7 mRNA assay. The primary endpoint was histologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+); the secondary endpoint was grade 3 or worse (CIN3+). We estimated marker positivity, triage referral, histology-verified CIN2 + capture, platform concordance, and inverse-probability-weighting (IPW)-corrected diagnostic accuracy.

Results

HR-HPV positivity was lower in AYA than non-AYA women on both platforms (DNA 15.7% vs 18.7%; mRNA 8.3% vs 12.8%; both $p < 0.001$). Among verified women, CIN2 + prevalence was similar (8.8% vs 8.5%), whereas CIN3 + was lower in AYA women (1.7% vs 3.0%; odds ratio 0.56, 95% confidence interval 0.35–0.89). In AYA co-tested women, cytology triage of mRNA-positive women referred 4.0% and captured 73.4% of verified CIN2+; the DNA pathway referred 6.1% and captured 68.8%. Genotype-only triage minimized referrals but missed many verified CIN2 + cases. Among 864 dual-tested women, platform agreement was moderate ($\kappa = 0.42$). Naive HR-HPV sensitivity of 82–87% fell to 43–51% after IPW correction.

Conclusions

AYA women did not have a higher verified CIN2 + burden and had fewer CIN3 + lesions than older women. Cytology-directed triage appeared to reduce colposcopy compared with direct referral. The apparent mRNA pathway advantage should be interpreted cautiously because platform assignment was non-random and mostly non-overlapping. Paired prospective studies with complete verification and follow-up are needed.

Background

Cervical cancer remains a major public-health challenge, and the World Health Organization has called for elimination through vaccination, screening, and treatment [1]. China carries a substantial cervical-cancer burden, while HR-HPV infection and cervical cancer risk vary by region, age, screening access, and vaccination uptake [2].

Cervical screening in adolescent and young adult (AYA) women, here defined as 15–39 years in line with AYA oncology conventions, raises a specific clinical and public-health dilemma. HR-HPV infection is common in younger women, but most infections are transient. A strategy that refers every HR-HPV-positive young woman directly to colposcopy may increase anxiety, cost, clinic workload, and potential overtreatment, particularly when the immediate risk of CIN3 + is low [3–7].

Two broad classes of HR-HPV tests are used in routine Chinese hospital screening [8–10]. HPV DNA assays detect viral genetic material and typically provide genotype information, whereas HPV E6/E7 mRNA assays detect oncogene transcription and may be more specific for clinically relevant infection [11–14]. Evidence comparing these approaches in routine AYA screening is limited, and direct comparisons are difficult when platforms are used in different clinical workflows.

Triage of HR-HPV-positive women by genotype or reflex cytology is central to reducing unnecessary colposcopy while preserving detection of clinically important precancer [15–18]. Other risk markers, including methylation and viral load, may further refine risk stratification [19, 20], and HPV16/18 genotyping has also been evaluated as a triage approach in young women [21]. We used two years of routine screening data from a large tertiary hospital in Henan, China, to characterize age-specific HR-HPV positivity, cytology, and histological outcomes; estimate PPV for key triage markers; compare colposcopy referral and CIN2 + detection under clinically realistic triage strategies within each platform; and quantify the effect of partial verification bias on diagnostic accuracy estimates.

Methods

Study design and setting

This was a single-centre, retrospective, cross-sectional study of women who underwent opportunistic cervical cancer screening at The Third Affiliated Hospital of Zhengzhou University, Henan Province, China, between January 2023 and December 2024. The diagnostic-accuracy components were prepared in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD), and the observational components followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations.

Study population and grouping

All screening encounters during the study period were eligible. Age was quality-controlled by removing biologically implausible or unclassifiable values. Women were assigned to mutually exclusive age

groups: AYA (15-39 years) and non-AYA (≥ 40 years). Because cervical cytology was available for nearly all screened women, the primary analytic cohort for screening and triage analyses comprised women with a valid cytology result.

HR-HPV testing platforms

HR-HPV status was determined using one of two routine laboratory platforms. For the DNA platform, HPV genotyping was performed using a PCR-based 23-genotype assay (Kaineng HPV Genotyping Kit; Kaineng Biotechnology, China), which detects 14 high-risk genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), 6 low-risk genotypes (HPV6, 11, 42, 43, 44, and 81), and 3 other genotypes (HPV53, 73, and 82) in a single assay. HPV mRNA testing used the Hologic Aptima HPV E6/E7 mRNA method, with HPV16 and HPV18/45 genotyping where available. Assays were performed and interpreted according to manufacturer instructions by the hospital's accredited laboratory. The two platforms were mutually exclusive for most women and were embedded in routine clinical workflows.

Cervical cytology and histology

Liquid-based cervical cytology was classified according to the Bethesda system. Cytological abnormality was defined at two thresholds: $\geq$ ASC-US and $\geq$ ASC-H. Cytology interpretation followed routine departmental quality-control procedures with double sign-out; difficult or discordant cases were reviewed in departmental consultation.

The reference standard was cervical histology obtained at colposcopy-directed biopsy or excision. Histological diagnoses were made within the routine pathology workflow using double sign-out, with departmental consultation for difficult cases. The primary endpoint was CIN2+ (CIN2, CIN3, adenocarcinoma in situ, or invasive cervical cancer), and the secondary endpoint was CIN3+.

Triage strategy definitions

Within each platform, we modelled three clinically realistic triage strategies among women with both an HPV result and cytology: (1) HPV primary referral, in which all HR-HPV-positive women would be referred to colposcopy; (2) genotype-directed referral, in which only HPV16/18 positive women on the DNA platform or HPV16/18-45 positive women on the mRNA platform would be referred; and (3) cytology-directed referral, in which HR-HPV-positive women would be referred only if concurrent cytology was $\geq$ ASC-US. For each strategy we calculated the colposcopy referral rate and the proportion of histology-verified CIN2+ cases that would have been referred, together with the number of verified CIN2+ cases missed.

Statistical analysis

Categorical variables are summarized as counts and percentages and were compared using the chi-square test or Fisher's exact test where appropriate. Between-group associations are expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Marker positivity and PPV for CIN2+ were estimated

with binomial 95% CIs. Agreement between the HPV DNA and HPV mRNA platforms among dual-tested women was quantified using overall agreement, positive and negative percentage agreement, and Cohen's kappa with 95% CIs. Two-sided $p < 0.05$ was considered statistically significant. Analyses were performed in Python using pandas, SciPy, and statsmodels.

OpenAI Codex was used as an assistive tool for data-analysis workflow checking and English-language polishing. The authors independently reviewed and verified all analytical outputs, interpretations, and manuscript revisions, and take full responsibility for the final content.

Correction for partial verification bias

Histological verification in routine screening is not random and occurs mainly among women with abnormal screening results. Naive estimates of diagnostic accuracy are therefore vulnerable to partial verification bias [22, 23]. We used inverse-probability weighting (IPW) to estimate diagnostic accuracy after accounting for differential verification [24-26]. Within each age group, women were stratified by cytology tier and HR-HPV status, and each verified woman was weighted by the inverse of the stratum-specific probability of verification. Corrected sensitivity, specificity, PPV, and NPV with 95% CIs were obtained by bootstrap resampling with 1,000 replicates.

Results

Cohort characteristics and platform distribution

Of 33,224 women screened during 2023-2024, 38 with invalid or missing age were excluded, yielding 33,186 age-classified women (AYA 21,206; non-AYA 11,980; Fig. 1). The analytic cohort comprised 32,737 women with valid cytology (AYA 20,926; non-AYA 11,811). AYA women were younger by definition (median 33 vs 48 years), and 71.8% of AYA women were aged 30-39 years (Table 1).

Test availability differed by age group. Among AYA women, 40.1% had HPV DNA testing and 53.5% had HPV E6/E7 mRNA testing, whereas non-AYA women more often had DNA testing (53.6%) than mRNA testing (40.9%). Only 436 AYA and 428 non-AYA women were tested on both platforms. Histological verification was available for 1,715 AYA women (8.1%) and 1,644 non-AYA women (13.7%).

Age-specific epidemiology

HR-HPV positivity showed a U-shaped age distribution, with higher positivity in the youngest and oldest age bands and a trough in the late twenties and thirties (Table 2, Fig. 2). On the DNA platform, HR-HPV positivity was lower in AYA than non-AYA women (15.7% vs 18.7%; OR 0.81, 95% CI 0.75-0.89; $p < 0.001$). The same pattern was observed on the mRNA platform (8.3% vs 12.8%; OR 0.62, 95% CI 0.56-0.69; $p < 0.001$). Cytological abnormality was also less frequent in AYA women at both $\geq$ ASC-US and $\geq$ ASC-H thresholds.

Among histologically verified women, CIN2+ prevalence was similar between age groups (AYA 8.8% vs non-AYA 8.5%; OR 1.05, 95% CI 0.82-1.33; $p=0.718$). CIN3+, however, was less frequent in AYA women (1.7% vs 3.0%; OR 0.56, 95% CI 0.35-0.89; $p=0.013$). Invasive cervical cancer was uncommon among verified AYA women (6 cases) and more frequent among verified non-AYA women (28 cases).

Positive predictive value of triage markers

PPV for CIN2+ increased when markers were restricted to higher-risk findings (Table 3, Fig. 3). Any HR-HPV positivity had low PPV (DNA: 15.4% in AYA and 14.9% in non-AYA; mRNA: 20.0% and 16.7%). Genotype-level positivity yielded higher PPV, especially for the mRNA 16/18-45 channel (47.1% in AYA and 32.3% in non-AYA). Cytology at the $\geq$ ASC-H threshold also showed higher PPV than $\geq$ ASC-US. These patterns indicate that more specific triage markers concentrated histologically verified CIN2+ more effectively than broad HR-HPV positivity alone.

Triage strategy trade-offs

Referral and detection trade-offs are shown in Table 4 and Fig. 4. Referring all HR-HPV-positive women detected the largest proportion of histology-verified CIN2+ but generated the highest colposcopy referral burden. Genotype-directed referral minimized colposcopy but missed many verified CIN2+ cases. Cytology-directed referral at $\geq$ ASC-US provided an intermediate strategy, reducing referrals while retaining more CIN2+ detection than genotype-only referral.

In AYA women, cytology-directed triage of mRNA-positive women referred 4.0% of co-tested women and captured 73.4% of histology-verified CIN2+ cases. The corresponding DNA-based strategy referred 6.1% and captured 68.8%. Similar directional patterns were observed in non-AYA women. These findings suggest a potentially more colposcopy-efficient mRNA pathway in this real-world setting, but the comparison is ecological because the platforms were applied to largely different groups of women.

Platform concordance and verification bias

Among the 864 women tested on both HPV platforms (Supplementary Table S2), overall agreement for HR-HPV status was 77.2%, with moderate agreement by Cohen's kappa (0.42, 95% CI 0.35-0.49). Positive percentage agreement was 49.2% and negative percentage agreement was 89.4%, using the DNA platform as the reference. Agreement was similar in AYA and non-AYA women, supporting the conclusion that the two assays should not be treated as interchangeable.

Histological verification was concentrated among screen-positive women (Fig. 5). Naive HR-HPV sensitivity exceeded 80% in both age groups but fell after IPW correction to 43.2% in AYA women and 50.5% in non-AYA women. Specificity increased after correction. Detailed corrected accuracy estimates are provided in Supplementary Table S1. These findings confirm substantial partial verification bias in routine screening data and show that naive diagnostic accuracy estimates should be interpreted cautiously.

Discussion

In this large real-world screening cohort from Henan, AYA women had lower overall marker positivity than older women on both HPV platforms and lower high-grade cytology, while the burden of histology-verified CIN2 + was similar and CIN3 + was lower. These findings argue against a uniformly more aggressive colposcopy policy for HR-HPV-positive AYA women and support the public-health value of triage.

The most clinically relevant pattern was the referral-detection trade-off. Direct referral of all HR-HPV-positive women maximized verified CIN2 + capture but required substantially more colposcopy. Genotype-only referral was highly colposcopy-sparing but missed many verified CIN2 + cases. Cytology-directed referral at the ASC-US threshold appeared to provide a more balanced strategy, particularly in younger women where avoiding unnecessary colposcopy and potential overtreatment is important.

The observed mRNA pathway had lower referral rates than the DNA pathway with similar or higher capture of histology-verified CIN2 + in the modeled strategies. This finding is biologically plausible because E6/E7 mRNA testing reflects oncogene transcription rather than viral DNA presence [11–14, 27–31]. However, this study does not establish direct superiority of the mRNA platform. Platform assignment was not randomized, and most women received only one platform. The moderate agreement in dual-tested women further shows that the assays capture related but non-identical signals. The platform comparison should therefore be read as hypothesis-generating evidence that warrants paired prospective evaluation.

The verification-bias analysis is important for interpreting all routine screening studies of this kind. Because histology was obtained mainly in screen-positive women, naive sensitivity and specificity were distorted. After IPW correction, apparent HR-HPV sensitivity fell markedly and specificity rose. This does not negate the clinical value of HPV testing; rather, it shows that diagnostic-accuracy estimates based only on verified women can be misleading unless verification mechanisms are addressed.

For screening programmes serving young women in China and similar settings [8–10, 17], the findings support a cautious, triage-based approach. Where an mRNA platform is available, cytology-directed triage of mRNA-positive women may help reduce colposcopy burden. Where DNA testing is used, cytology triage remains preferable to direct referral of all positives. Further work should evaluate age-specific risk thresholds, CIN3 + outcomes, HPV persistence, vaccination status, molecular biomarkers, and long-term safety [19, 20, 32–34].

Limitations

Several limitations should be considered. First, this was a single-centre retrospective study of opportunistically screened women, which may limit generalizability. Second, the HPV DNA and mRNA platforms were applied to largely non-overlapping populations, so platform comparisons are ecological rather than paired or randomized. Residual confounding by clinical workflow, provider preference, patient

characteristics, or calendar time cannot be excluded. Third, histological verification was incomplete and selective. Although IPW was used to examine diagnostic accuracy, unmeasured determinants of verification may remain. Fourth, triage detection rates were based on histology-verified CIN2 + and should not be interpreted as complete population sensitivity. Fifth, vaccination status, prior screening history, HPV persistence, and longitudinal outcomes were not available. Finally, the AYA category includes a wide age range; the majority of AYA participants were aged 30–39 years, and findings may not generalize equally to adolescents or women in their early twenties.

Conclusions

In this real-world cohort, AYA women did not have a higher verified CIN2 + burden than older women and had fewer CIN3 + lesions, supporting triage rather than routine direct colposcopy referral for all HR-HPV-positive young women. Cytology-directed triage provided a pragmatic balance between colposcopy referral and verified CIN2 + detection. The mRNA pathway showed a favourable referral profile in ecological comparisons, but paired prospective studies with complete verification, longitudinal follow-up, and age-stratified CIN3 + outcomes are needed before strong platform-specific recommendations can be made.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University (2025-345-01). Because this was a retrospective analysis of de-identified routine clinical data, the requirement for individual informed consent was waived by the review board.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available because they contain institutional clinical records, but de-identified aggregate data may be available from the corresponding author on reasonable request and subject to institutional approval.

Competing interests

The authors declare that they have no competing interests.

Funding

This study received funding from the Henan Provincial Medical Science and Technology Research Program Joint Construction Project (grant number LHGJ20250375).

Authors' contributions

Shuailiang Xin and Yongzhen Guo conceived the study, contributed to study design, data curation, diagnostic validation, data analysis, interpretation of results, and drafted the manuscript. Tong Xue, Yunling Li, and Mengke Qin contributed to data collection, clinical and laboratory record verification, data curation, and manuscript revision. Yixuan Liu contributed to statistical analysis, figure and table preparation, and interpretation of analytical results. Wei Zhang conceived and supervised the study, contributed to methodology, result interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Dorji Thinley; Tshomo Ugyen; Gyamtsho Sonam; Tamang Saran Tenzin. Wangmo Sangay; Pongpirul Krit. Gender-neutral HPV elimination, cervical cancer screening, and treatment: Experience from Bhutan. *Int J Gynaecol Obstet*. 2021. 10.1002/ijgo.13728.
2. Yang X. Qi Shiyuan; Dai Liyan; Ye Qingjian; Li Xiaomao. Trends in HPV-positive cervical cancer prevalence: a retrospective study from 2013 to 2020. *Virol J*. 2025. 10.1186/s12985-025-02830-7.
3. Quan J et al. Chen Qi; Lan Linchen; Zhang Bin; Hu Yanyun; Zhao Fei,. Type- and age-specific natural history of high-risk human papillomavirus infections in healthy women: A prospective cohort study in China. *International journal of cancer*. 2026. 10.1002/ijc.70337
4. Wei Feixue; Xia Ningshao; Ocampo Rebeca, Goodman Marc T, Hessel Nancy A, Grinsztejn, Beatriz, et al. Age-Specific Prevalence of Anal and Cervical Human Papillomavirus Infection and High-Grade Lesions in 11 177 Women by Human Immunodeficiency Virus Status: A Collaborative Pooled Analysis of 26 Studies. *J Infect Dis*. 2023. 10.1093/infdis/jiac108.
5. Aro Karoliina; Nieminen Pekka; Louvanto Karolina; Jakobsson Maija; Virtanen Seppo; Lehtinen Matti, et al. Age-specific HPV type distribution in high-grade cervical disease in screened and unvaccinated women. *Gynecologic oncology et al*. 2019. 10.1016/j.ygyno.2019.05.024
6. Kjær Susanne K, Munk C. Junge Jette; Iftner Thomas. Carcinogenic HPV prevalence and age-specific type distribution in 40,382 women with normal cervical cytology, ASCUS/LSIL, HSIL, or cervical cancer: what is the potential for prevention? *Cancer causes & control : CCC*. 2013. 10.1007/s10552-013-0320-z

7. Haidopoulos D, Voulgaris Z, Protopapas A, Rodolakis A, Vlachos G, Tsetsa P, et al. Cervical intraepithelial neoplasia in young women. *J Obstet Gynaecol J Inst Obstet Gynaecol*. 2007. 10.1080/01443610701612615.
8. Han S, Lin M et al. Liu Mengyu; Wu Shiwan; Guo Pi; Guo Jiubiao,. Prevalence, trends, and geographic distribution of human papillomavirus infection in Chinese women: a summative analysis of 2,728,321 cases. *BMC medicine*. 2025. 10.1186/s12916-025-03975-6
9. Zhong H et al. Pan Wenwei; Chen Binbin; Gu Jiamin; Liang Yu; Sun Guoli,. A real-world, cross-sectional, and longitudinal study on high-risk human papillomavirus genotype distribution in 31,942 women in Dongguan, China. *Frontiers in public health*. 2024. 10.3389/fpubh.2024.1409030
10. Mo B et al. Ye Yuanxin; Yu Maowen; Tong Xianli; Cao Hongmei; Du Chunmei,. Prevalence and genotype distribution of HPV combined with cervical pathological results in women from Sichuan, China: A cross-sectional study based on post-vaccination period 2019 to 2023. *Cancer medicine*. 2024. 10.1002/cam4.70148
11. White Christine; Reynolds Stephen; Murphy Katherine; Keegan Helen; Naik Padma; O'Brien Roisin, et al. Performance of the HPV E6/E7 mRNA Aptima HPV assay combined with partial genotyping compared with the HPV DNA Cobas 4800 HPV test for use in primary screening: Results from the CERVIVA HPV primary screening study in Ireland. *International journal of cancer et al*. 2023. 10.1002/ijc.34685
12. Giorgi Rossi Paolo; Carozzi Francesca; Ronco Guglielmo; Allia Elena; Bisanzi Simonetta; Gillio-Tos Anna, et al. p16/ki67 and E6/E7 mRNA Accuracy and Prognostic Value in Triaging HPV DNA-Positive Women. *Journal of the National Cancer Institute et al*. 2021. 10.1093/jnci/djaa105
13. Pi R, Li T, Zhang H et al. Zhou Hang; Yang Yuan; Dai Yu,. The Distribution of HR-HPV E6/E7 DNA and mRNA by Histological Grade and the Clinical Performance for Detection of Cervical Cancer and Precancer. *Journal of medical virology*. 2024. 10.1002/jmv.70026
14. Duvlis Sotirija; Popovska-Jankovic Katerina; Arsova Zorica Sarafinovska. Memeti Shaban; Popeska Zaneta; Plaseska-Karanfilska Dijana. HPV E6/E7 mRNA versus HPV DNA biomarker in cervical cancer screening of a group of Macedonian women. *J Med Virol*. 2015. 10.1002/jmv.24199.
15. Dun Changchang; Yuan Meiwen, Zhao X, Hu Shangying. Arbyn Marc; Zhao Fanghui. Clinical evaluation of primary human papillomavirus (HPV) testing with extended HPV genotyping triage for cervical cancer screening: A pooled analysis of individual patient data from nine population-based cervical cancer screening studies from China. *Cancer Med*. 2024. 10.1002/cam4.7316.
16. Rezhake Remila; Abuduxikuer Guzhanuer; Abudurexiti Guligeina; Zhuo Qian; Muhetaer Kadeliya; Abulimiti Tangnuer, et al. Evaluation of the multiple HPV-based et al. screen and triage algorithms in real-world settings of rural China. *Cancer biology & medicine*. 2025. 10.20892/j.issn.2095-3941.2025.0384
17. Yao Boshuang; Peng Jieru, Song W, Yang L, Zhang M, Wu, Xia, et al. Real-world effectiveness of cytology and HPV-based screening strategy in cervical cancer screening: A cross-sectional population-based study in Chengdu, China. *PLoS ONE*. 2024. 10.1371/journal.pone.0299651.

18. Li J, Yao C, Zhao C, Li M, Li L, Lin, Zhixin, et al. Distribution and extended triage of high-risk human papillomavirus genotyping based on vaginal self-sampling. *BMJ public health*. 2025. 10.1136/bmjph-2024-001565.
19. Fan X, Guo H, Wang S, Xiangwen P. HPV16/18 Genotyping Combined With PAX1 Methylation Triage Reduces Immediate Colposcopy While Improving CIN3 + Detection: A Real-World Study of 3,233 Chinese Women. *Cancer Control*. 2026. 10.1177/10732748261442617.
20. Chen YQ, Huang QM, Hong ML, Zhu YQ, Gao YL, Chen LY et al. [Value of high-risk HPV viral load in cervical cancer screening and triage: a real world retrospective study based on cervical cancer screening program in Quanzhou, China]. *Zhonghua fu chan ke za zhi*. 2025. 10.3760/cma.j.cn112141-20241210-00657
21. McKenna M, McMenamin M, McDowell A. HPV16 and HPV18 genotyping triage in young women with borderline cytology or mild dyskaryosis: effect of age on genotype-specific risk of high-grade CIN. *Cytopathol : official J Br Soc Clin Cytol*. 2016. 10.1111/cyt.12316.
22. Kohn MA. Studies of diagnostic test accuracy: Partial verification bias and test result-based sampling. *J Clin Epidemiol*. 2022. 10.1016/j.jclinepi.2022.01.022.
23. Arifin Wan Nor; Yusof Umi Kalsom. Correcting for partial verification bias in diagnostic accuracy studies: A tutorial using R. *Statistics in medicine*. 2022. 10.1002/sim.9311
24. Day E, Eldred-Evans D, Prevost A, Toby; Ahmed Hashim U, Fiorentino Francesca. Adjusting for verification bias in diagnostic accuracy measures when comparing multiple screening tests - an application to the IP1-PROSTAGRAM study. *BMC Med Res Methodol*. 2022. 10.1186/s12874-021-01481-w.
25. Arifin Wan Nor; Yusof Umi Kalsom. Partial verification bias correction using scaled inverse probability resampling for binary diagnostic tests. *PLoS ONE*. 2025. 10.1371/journal.pone.0321440.
26. Arifin Wan Nor; Yusof Umi Kalsom. Partial Verification Bias Correction Using Inverse Probability Bootstrap Sampling for Binary Diagnostic Tests. *Diagnostics (Basel Switzerland)*. 2022. 10.3390/diagnostics12112839.
27. Giorgi Rossi Paolo; Ronco Guglielmo; Mancuso Pamela; Carozzi Francesca; Allia Elena; Bisanzi Simonetta, et al. Performance of HPV E6/E7 mRNA assay as primary screening test: Results from the NTCC2 trial. *International journal of cancer et al*. 2022. 10.1002/ijc.34120
28. Sørbye S, Falang BM, Antonsen M, Mortensen E, Genotype-Specific HPV. mRNA Triage Improves CIN2 + Detection Efficiency Compared to Cytology: A Population-Based Study of HPV DNA-Positive Women. *Pathogens (Basel, Switzerland)*. 2025. 10.3390/pathogens14080749
29. Sørbye Sveinung Wergeland; Falang Bente Marie. Antonsen Mona; Richardsen Elin. Genotype-Specific HPV mRNA Triage Improves Colposcopy Efficiency Compared with Cytology and ATHENA-Derived Triage: A Population-Based Study of HPV DNA-Positive Women. *Pathogens (Basel, Switzerland)*. 2026. 10.3390/pathogens15060584
30. Flores Carlos Eduardo Aranda; Falang Bente Marie. Enhancing Cervical Cancer Screening with 7-Type HPV mRNA E6/E7 Testing on Self-Collected Samples: Multicentric Insights from Mexico.

- Cancers. 2024. 10.3390/cancers16132485. Gómez-Laguna Laura; Gutiérrez Guillermo Gómez; León Jorge Miguel Ortiz; Uribe Miguel.
31. Pankaj Sangeeta; Rani Jyotsna; Kumari Pratibha; Abhilashi Kavya; Choudhary Vijayanand; Kumari Satya, et al. Efficacy of HPV E6/E7 mRNA assay, HPV DNA test and cytology in detection of high grade cervical lesions and invasive cancer at a tertiary care center in India. *Malawi medical journal : the journal of Medical Association of Malawi* et al. 2024. 10.4314/mmj.v36i2.9
32. Eriksen Dina O, Lycke Kathrine D, Kahlert J, Ostensfeld Eva B, Jensen Pernille T, Wentzensen N, et al. Active surveillance of cervical intraepithelial neoplasia grade 2 is not associated with an increased risk of noncervical anogenital human papillomavirus-related cancer and precancer: a population-based cohort study. *Am J Obstet Gynecol*. 2025. 10.1016/j.ajog.2025.05.039.
33. Li B, Guo R, Lai Tianjiao. Qiao Long; Fu Hanlin. The application of PAX1 methylation detection and HPV E6/E7 mRNA detection in cervical cancer screening. *J Obstet Gynaecol Res*. 2021. 10.1111/jog.14869.
34. Parida P. Baburaj Gayathri; Rao Mahadev; Lewis Shirley; Damerla Rama Rao. Circulating cell-free DNA as a diagnostic and prognostic marker for cervical cancer. *Int J Gynecol Cancer*. 2024. 10.1136/ijgc-2023-004873.

Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

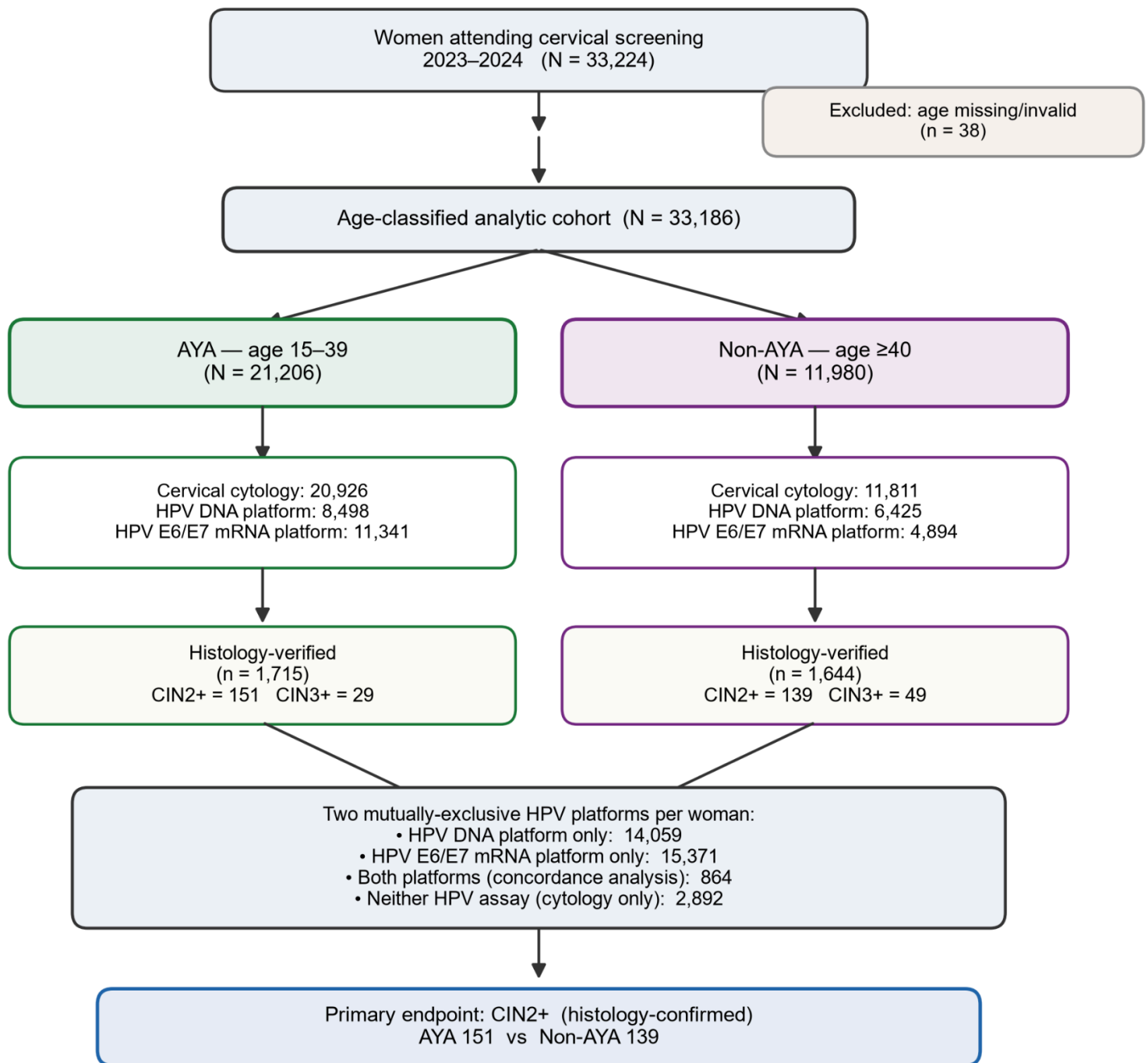


Figure 1

Study flow (STARD). Flow of participants from enrolment to the primary analysis.

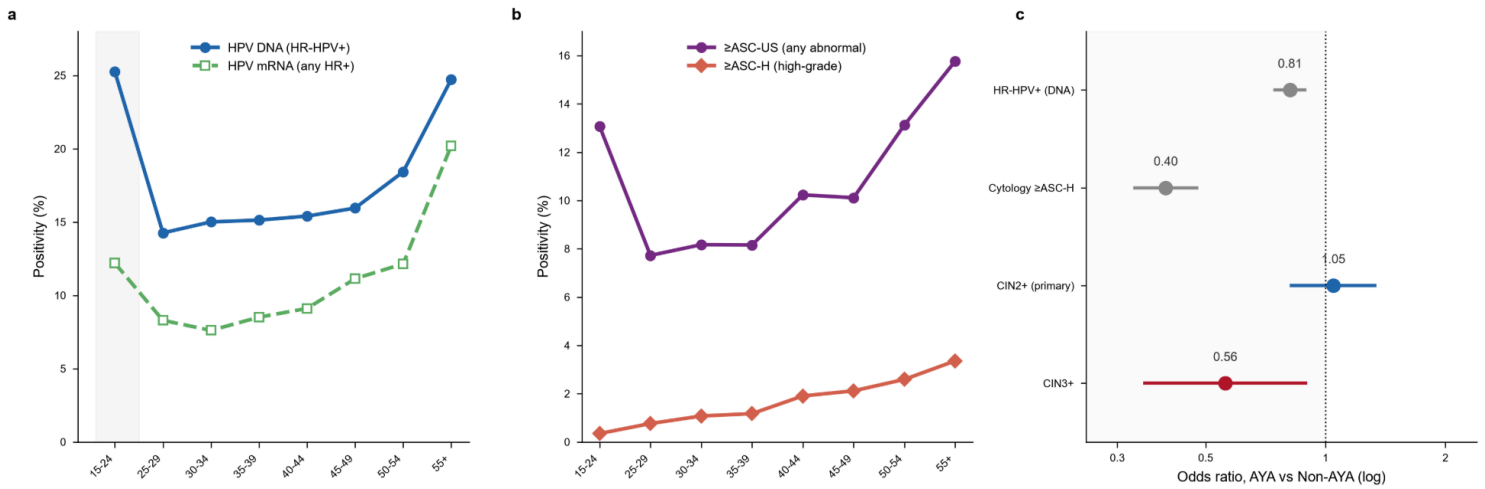


Figure 2

Age-related epidemiology of infection, cytology, and histologic outcome. (a) HR-HPV positivity by age band, shown separately for the HPV DNA platform (solid) and the HPV E6/E7 mRNA platform (dashed). (b) Prevalence of any cytological abnormality ($\geq$ ASC-US) and high-grade cytology ($\geq$ ASC-H) by age band. (c) Age-group contrasts expressed as odds ratios (AYA vs non-AYA, log scale) with 95% confidence intervals.

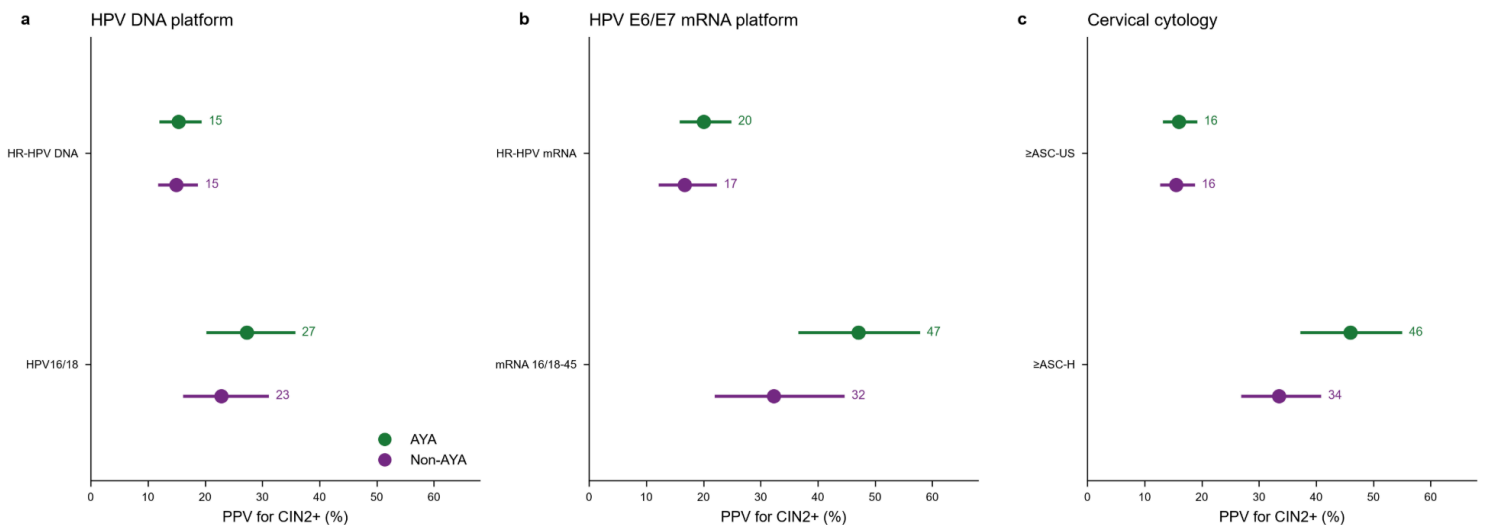


Figure 3

Positive predictive value for CIN2+ rises from screen-positivity to genotype-level triage. Point estimates with 95% confidence intervals (Wilson) show the positive predictive value of each marker for histologically confirmed CIN2+ within the histology-verified population, separately for AYA and non-AYA women. (a) HPV DNA platform: any HR-HPV positive vs HPV16/18 positive. (b) HPV E6/E7 mRNA platform: any HR-HPV positive vs the HPV16/18-45 channel. (c) Cervical cytology: $\geq$ ASC-US vs $\geq$ ASC-H.

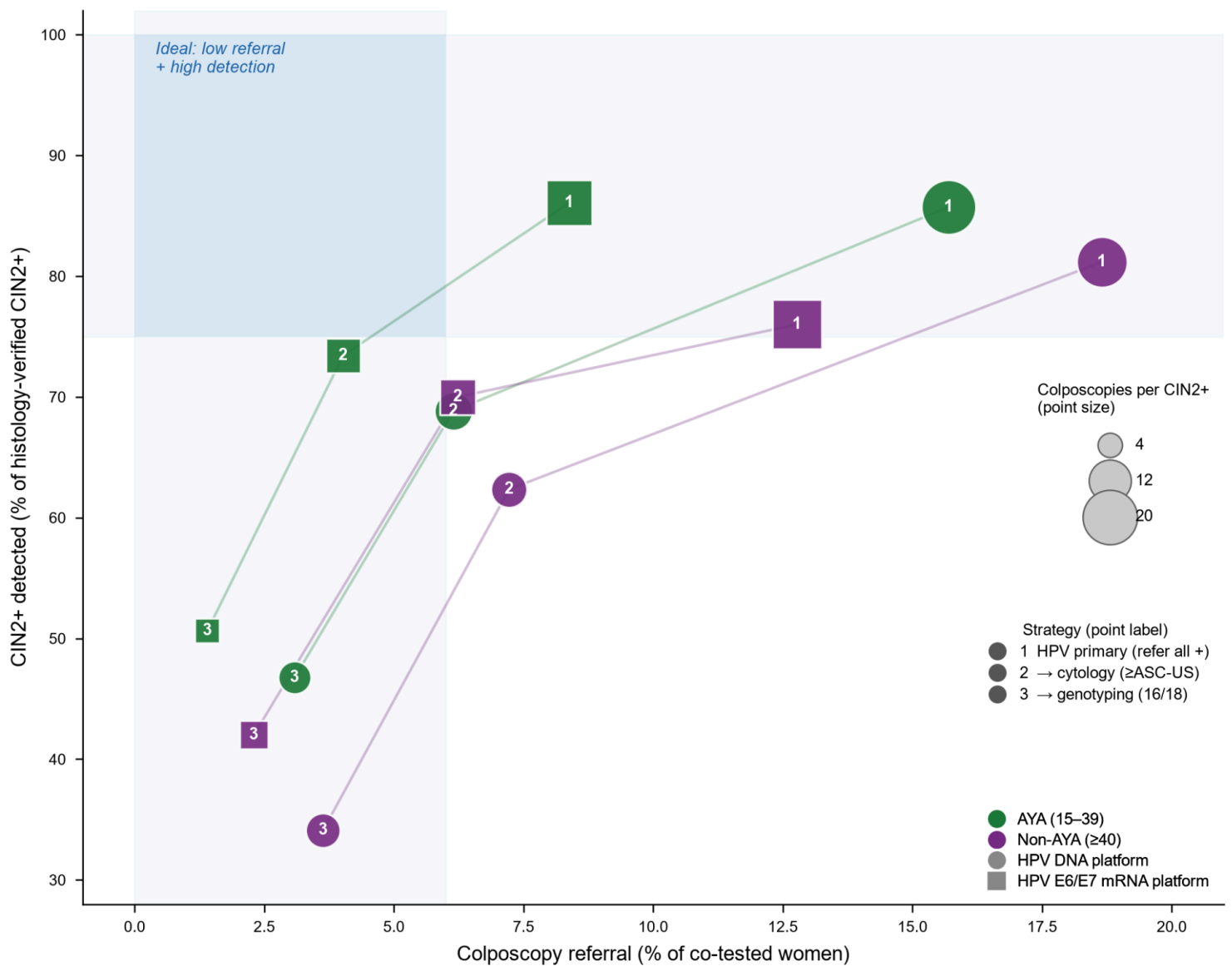


Figure 4

Triage strategies trade colposcopy referral against histology-verified CIN2+ capture. Each point is one triage strategy in the co-tested population (women with both an HPV result and cytology), plotted by colposcopy referral rate (x-axis, % of co-tested women) against the proportion of histology-verified CIN2+ cases captured (y-axis). Colour denotes age group and marker shape denotes platform (circle, HPV DNA platform; square, HPV E6/E7 mRNA platform). Point labels index the strategy: 1 = HPV primary, refer all positives; 2 = HPV positive followed by cytology triage (refer ≥ASC-US); 3 = HPV positive followed by genotyping triage (refer HPV16/18 on the DNA platform or HPV16/18-45 on the mRNA platform). Lines connect the three strategies within each group-platform trajectory. Point size is proportional to colposcopies performed per CIN2+ captured (smaller = more efficient). The upper-left shaded region marks the ideal zone of lower referral and higher verified CIN2+ capture.

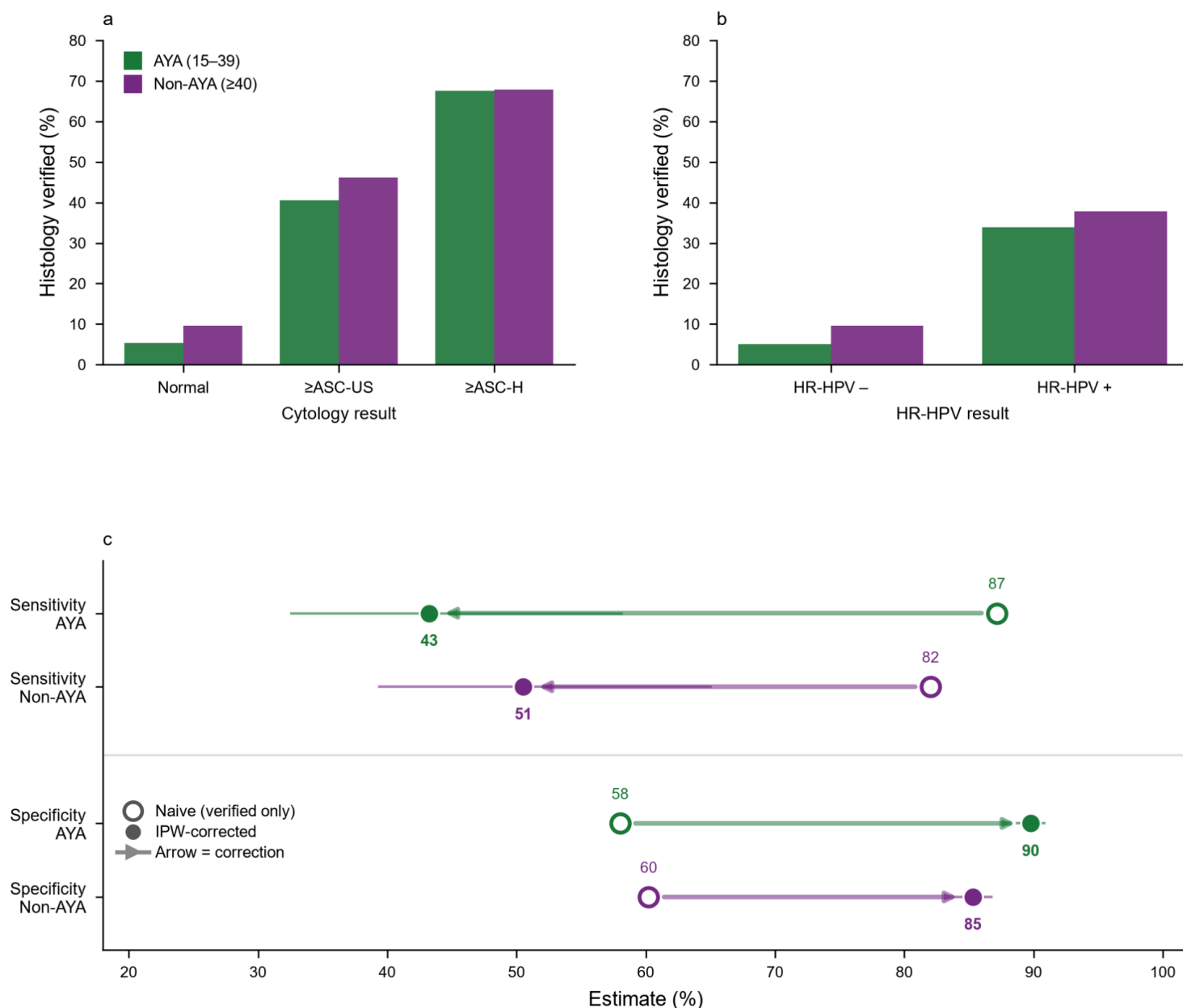


Figure 5

Verification bias inflates apparent HR-HPV sensitivity, and inverse-probability weighting corrects it in both age groups.(a) Proportion of women receiving histologic verification by cytology result and (b) by HR-HPV status, for AYA and non-AYA women. (c) Sensitivity and specificity of HR-HPV for CIN2+, comparing naive estimates restricted to verified women with inverse-probability-weighted (IPW) estimates; arrows show the direction of correction and whiskers are 95% bootstrap confidence intervals for the IPW estimate. IPW weights were the inverse of the stratum-specific verification probability, with strata defined by cytology tier and HR-HPV status within each age group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)
- [Tables.docx](#)