

Single Primary Targeted Biopsy Versus Targeted Plus Systematic Four Quadrant Biopsies With Or Without Endocervical Curettage For Detecting CIN2 Plus At Colposcopy: A Paired Diagnostic Accuracy Study

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ABSTRACT

Targeted colposcopic biopsy has imperfect sensitivity for cervical precancer, with a clinically important risk of missing CIN2 plus. This study compared a prespecified single primary targeted biopsy strategy with strategies that add systematic four quadrant biopsies and endocervical curettage.

This paired diagnostic accuracy study evaluated nested index strategies within the same patients. Strategy A was the prespecified primary targeted biopsy from the most suspicious lesion. Strategy B1 included all targeted biopsies plus systematic four quadrant biopsies. Strategy B2 included B1 plus endocervical curettage. Reference standard was excision histology or 12 month follow up verification. The primary endpoint was the paired sensitivity difference for CIN2 plus.

Among 268 reference standard evaluable patients, reference CIN2 plus prevalence was 61 of 268 (22.8%). Sensitivity for CIN2 plus was 75.4% for Strategy A (46 of 61), 91.8% for Strategy B1 (56 of 61), and 95.1% for Strategy B2 (58 of 61). Compared with Strategy A, sensitivity increased by 16.4 percentage points for Strategy B1 ($p=0.00195$) and by 19.7 percentage points for Strategy B2 ($p=0.00049$). Endocervical curettage contributed an additional 2 detections among reference positive patients (3.3 percentage points; $p=0.50$). Specificity for CIN2 minus was 99.5% for all strategies. Patient based number needed to sample was 26.2 for four quadrant biopsies and 67 for endocervical curettage.

Adding systematic four quadrant biopsies to targeted biopsy substantially increased CIN2 plus detection without an observed specificity penalty in this dataset. Endocervical curettage provided a smaller incremental yield overall, supporting selective use in higher risk profiles.

Keywords: colposcopy, cervical intraepithelial neoplasia, targeted biopsy, endocervical curettage, diagnostic accuracy

Introduction

Cervical cancer prevention relies on effective screening, risk stratified triage, and accurate diagnostic evaluation of screen positive individuals. Current guidance endorses risk based screening and management pathways using cytology and high risk human papillomavirus testing, with colposcopy as the central diagnostic procedure for many abnormal results. (1,2) Even in settings with increasing vaccination coverage, modelling work indicates that screening quality

and downstream diagnostic performance remain critical for reducing cervical cancer burden. (3)

Colposcopy performance is constrained by lesion heterogeneity, variability in transformation zone visualization, and sampling error. A systematic review and meta-analysis of colposcopic accuracy using the 2011 IFCPC terminology underscores substantial variability in diagnostic performance and supports the view that biopsy practice, rather than colposcopic impression alone, determines detection yield. (4)

Evidence from prospective colposcopy studies demonstrates that taking multiple biopsies

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increases detection of cervical precancer compared with fewer biopsies (5). ASCCP colposcopy standards and related consensus recommendations emphasize standardized assessment, documentation, quality improvement, and risk aligned biopsy practice, including attention to transformation zone type and squamocolumnar junction visibility. (6-11)

Beyond multiple targeted biopsies, systematic four quadrant sampling and endocervical curettage may improve detection of lesions that are small, multifocal, or endocervical. Guidance for endocervical curettage supports selective use in higher risk profiles and in settings of incomplete squamocolumnar junction visualization, while avoiding use in pregnancy. (12) The 2019 ASCCP risk based management guidelines and supporting risk estimation methods provide a framework that shapes the risk spectrum of patients referred to colposcopy, including the role of partial genotyping and triage. (13-16) Prospective work on risk based colposcopy provides a clinical rationale for aligning sampling intensity with patient risk. (17)

Any strategy that increases detection should be interpreted alongside potential harms. CIN2 has meaningful regression potential under surveillance in selected patients, and recent evidence and consensus statements support active surveillance pathways. (18-20) Excisional treatment is also associated with adverse obstetric outcomes that increase with cone depth, strengthening the need to balance diagnostic intensity against downstream consequences. (21) European position statements similarly emphasize optimizing detection of clinically meaningful lesions while limiting unnecessary procedures. (22)

The primary aim of this study was to compare, within the same patients, the sensitivity of a prespecified single primary targeted biopsy strategy versus strategies that add systematic four quadrant biopsies without endocervical curettage and with endocervical curettage for reference confirmed CIN2 plus. Secondary aims included evaluation of CIN3 plus, the incremental yield attributable to four quadrant biopsies and endocervical curettage, number needed to sample, operational over-detection proxies, prespecified subgroup summaries, and sensitivity analysis restricted to excision verified outcomes. Reporting followed STARD 2015 recommendations for diagnostic accuracy studies. (23)

Materials and Methods

Study Design and Setting: This was a paired diagnostic accuracy study with nested index strategies evaluated against a composite reference standard. The study was conducted in a colposcopy clinic setting. The dataset included five colposcopists and three pathologists. The analysis was prespecified and reported in accordance with STARD 2015. (23)

Participants and Analysis Populations: Patients were referred for colposcopy based on abnormal cytology, high risk human papillomavirus results, or both. Analysis populations were defined to support transparent reporting and to reduce ambiguity in paired comparisons. Participant flow and analysis populations are summarized.

Index Test Strategies: Strategy A was defined as the prespecified single primary targeted biopsy from the most suspicious lesion identified at the time of colposcopy. If multiple targeted biopsies were taken clinically, only the prespecified primary target biopsy contributed to Strategy A.

Strategy B1 included all targeted biopsies and systematic four quadrant biopsies, excluding endocervical curettage. Strategy B1 was positive if any targeted or four quadrant specimen showed CIN2 plus.

Strategy B2 included all Strategy B1 components plus endocervical curettage and was positive if any targeted, four quadrant, or endocervical curettage specimen showed CIN2 plus. Because Strategy A is contained within Strategies B1 and B2, A positive with B negative is structurally expected to be rare, and incremental yield is quantified by A negative with B positive discordant pairs.

Reference Standard and Verification Pathways: The reference standard was defined as the worst available outcome from excision histology (LEEP or conization) or 12 month follow up verification. Verification pathways were recorded per patient and summarized. Because not all patients underwent the same reference pathway, differential verification was addressed through a sensitivity analysis restricted to excision verified patients.

Variables and Data Structure: Data were stored in patient level and specimen level long format structures. Patient level variables included age, smoking status, immunosuppression, menopausal status, referral indication, cytology category, human papillomavirus results including HPV16 and HPV18, and standardized colposcopic findings such as transformation zone type,

squamocolumnar junction visibility, colposcopy adequacy, colposcopic impression, and acetowhite findings. Specimen level data included specimen type, location, adequacy, and histology category.

Outcomes: The primary outcome was reference confirmed CIN2 plus. The primary endpoint was the paired sensitivity difference for CIN2 plus comparing Strategy B1 versus Strategy A and Strategy B2 versus Strategy A among reference CIN2 plus patients. Secondary endpoints included paired specificity differences among reference CIN2 minus patients, predictive values, CIN3 plus performance, incremental yield attributable to four quadrant biopsies and endocervical curettage, patient based and specimen based number needed to sample, operational over-detection proxies, prespecified subgroup summaries, and excision only sensitivity analysis.

Statistical Analysis: Sensitivity, specificity, positive predictive value, and negative predictive value were calculated with 95 percent confidence intervals using Wilson methods. Paired comparisons of sensitivity among reference CIN2 plus patients and specificity among reference CIN2 minus patients used exact McNemar testing when discordant counts were small. Bootstrap resampling was used to estimate confidence intervals for paired sensitivity differences and predictive value differences. Model 1 assessed predictors of reference CIN2 plus using pre and peri colposcopy variables with cluster robust standard errors by colposcopist. Model 2 assessed predictors of incremental capture, defined as reference CIN2 plus cases missed by Strategy A but detected by Strategy B2, and was treated as exploratory given the limited number of events.

This study utilized existing clinical data from routine colposcopy practice. The sample size was not predetermined prospectively but was based on consecutive patients meeting inclusion criteria over the study period. Post-hoc power calculation was performed for the primary endpoint. With 61 reference CIN2+ patients and a paired sensitivity difference of 16.4 percentage points (Strategy B1 vs A), the study achieved >90% power to detect this clinically meaningful difference using exact paired McNemar testing at $\alpha=0.05$. For the comparison of Strategy B2 versus Strategy A (19.7 percentage point difference), power exceeded 95%.

Results

Participant flow and prevalence: Baseline and colposcopic characteristics of the reference

standard evaluable cohort are summarized in Table 1. Participant flow and reference verification pathways are shown in Figure 1 and Table 2. In the full dataset there were 300 records, with 294 procedure complete cases and 268 reference standard evaluable patients. Verification pathways included excision histology in 128 patients and 12-month verification in 140 patients. Reference confirmed CIN2 plus prevalence was 61 of 268 (22.8 percent) and reference confirmed CIN3 plus prevalence was 31 of 268 (11.6 percent).

Primary Endpoint: Paired Sensitivity For Cin2 Plus: Primary paired comparisons are summarized in Table 3 and illustrated in Figure 2. Among reference CIN2 plus patients ($n=61$), sensitivity of Strategy A was 75.4 percent (46 of 61). Sensitivity increased to 91.8 percent for Strategy B1 (56 of 61), corresponding to an absolute gain of 16.4 percentage points and 10 incremental detections (exact McNemar $p=0.00195$). Sensitivity increased to 95.1 percent for Strategy B2 (58 of 61), corresponding to an absolute gain of 19.7 percentage points and 12 incremental detections ($p=0.00049$).

The incremental yield attributable to endocervical curettage, comparing Strategy B2 versus Strategy B1, was 2 additional detections among reference positive patients (3.3 percentage points) and was not statistically significant in this dataset ($p=0.50$).

Specificity and Predictive Values: Overall diagnostic accuracy metrics are presented in Table 3 and displayed in Figure 3. Among reference CIN2 minus patients ($n=207$), specificity was 99.5 percent for Strategies A, B1, and B2, with no paired specificity differences detected. Positive predictive value remained high across strategies, while negative predictive value increased as false negative counts decreased under Strategies B1 and B2.

Incremental Yield, Number Needed To Sample, and Burden Proxies: Incremental yield and number needed to sample are summarized in Table 4 and visualized in Figure 4. Among reference CIN2 plus patients, incremental CIN2 plus yield attributable to systematic four quadrant biopsies were 10 of 61 (16.4 percent) and incremental yield attributable to endocervical curettage was 2 of 61 (3.3 percent). Patient based number needed to sample was 26.2 for four quadrant biopsies and 67 for endocervical curettage.

Sampling burden and operational over-detection proxies are also reported in Table 4. Among

Table 1: Baseline, Referral, and Colposcopic Characteristics of The Reference Standard Evaluable Cohort (n=268) and Stratified By Reference CIN2 Status

Characteristic	Overall	CIN2-	CIN2+
Panel A. Demographic and referral characteristics			
Age, years	36.0±8.1; median 35 [30–42]	36.1±8.0; median 35 [30–42]	35.6±8.4; median 35 [30–42]
Smoking: Never	147/268 (54.9%)	116/207 (56.0%)	31/61 (50.8%)
Smoking: Former	44/268 (16.4%)	32/207 (15.5%)	12/61 (19.7%)
Smoking: Current	77/268 (28.7%)	59/207 (28.5%)	18/61 (29.5%)
Immunosuppression: No	252/268 (94.0%)	194/207 (93.7%)	58/61 (95.1%)
Immunosuppression: Yes	16/268 (6.0%)	13/207 (6.3%)	3/61 (4.9%)
Menopausal status: Premenopausal	232/268 (86.6%)	178/207 (86.0%)	54/61 (88.5%)
Menopausal status: Perimenopausal	18/268 (6.7%)	16/207 (7.7%)	2/61 (3.3%)
Menopausal status: Postmenopausal	10/268 (3.7%)	6/207 (2.9%)	4/61 (6.6%)
Indication: Abnormal cytology	130/268 (48.5%)	119/207 (57.5%)	11/61 (18.0%)
Indication: HR-HPV positive	8/268 (3.0%)	6/207 (2.9%)	2/61 (3.3%)
Indication: Both	130/268 (48.5%)	82/207 (39.6%)	48/61 (78.7%)
Cytology: NILM	41/268 (15.3%)	39/207 (18.8%)	2/61 (3.3%)
Cytology: ASC-US	61/268 (22.8%)	53/207 (25.6%)	8/61 (13.1%)
Cytology: LSIL	56/268 (20.9%)	48/207 (23.2%)	8/61 (13.1%)
Cytology: ASC-H	19/268 (7.1%)	11/207 (5.3%)	8/61 (13.1%)
Cytology: HSIL	75/268 (28.0%)	47/207 (22.7%)	28/61 (45.9%)
Cytology: AGC	9/268 (3.4%)	5/207 (2.4%)	4/61 (6.6%)
Cytology: AIS	5/268 (1.9%)	2/207 (1.0%)	3/61 (4.9%)
Cytology: Other	2/268 (0.7%)	2/207 (1.0%)	0/61 (0.0%)
HR-HPV: Positive	138/268 (51.5%)	88/207 (42.5%)	50/61 (82.0%)
HR-HPV: Negative	130/268 (48.5%)	119/207 (57.5%)	11/61 (18.0%)
HPV16 positive	24/268 (9.0%)	11/207 (5.3%)	13/61 (21.3%)
HPV18 positive	11/268 (4.1%)	5/207 (2.4%)	6/61 (9.8%)
Other HR-HPV positive	120/268 (44.8%)	76/207 (36.7%)	44/61 (72.1%)
Multiple HR-HPV infection: No	241/268 (89.9%)	197/207 (95.2%)	44/61 (72.1%)
Multiple HR-HPV infection: Yes	18/268 (6.7%)	5/207 (2.4%)	13/61 (21.3%)
Panel B. Colposcopic characteristics			
Transformation zone: Type 1	67/268 (25.0%)	48/207 (23.2%)	19/61 (31.1%)
Transformation zone:	140/268 (52.2%)	112/207 (54.1%)	28/61 (45.9%)

Type 2			
Transformation zone:	61/268 (22.8%)	47/207 (22.7%)	14/61 (23.0%)
Type 3			
SCJ visibility: Fully visible	108/268 (40.3%)	84/207 (40.6%)	24/61 (39.3%)
SCJ visibility: Partially visible	98/268 (36.6%)	77/207 (37.2%)	21/61 (34.4%)
SCJ visibility: Not visible	62/268 (23.1%)	46/207 (22.2%)	16/61 (26.2%)
Colposcopy adequacy: Adequate	210/268 (78.4%)	164/207 (79.2%)	46/61 (75.4%)
Colposcopy adequacy: Inadequate	58/268 (21.6%)	43/207 (20.8%)	15/61 (24.6%)
Colposcopic impression: Normal	122/268 (45.5%)	120/207 (58.0%)	2/61 (3.3%)
Colposcopic impression: Low-grade	94/268 (35.1%)	78/207 (37.7%)	16/61 (26.2%)
Colposcopic impression: High-grade	49/268 (18.3%)	6/207 (2.9%)	43/61 (70.5%)
Colposcopic impression: Suspicious for cancer	3/268 (1.1%)	3/207 (1.4%)	0/61 (0.0%)
Lesion count	1.3±1.0; median 1 [1–2]	1.1±0.9; median 1 [0–2]	2.0±1.0; median 2 [1–3]
Quadrants involved (0–4)	1.5±1.1; median 1 [1–2]	1.2±0.9; median 1 [1–2]	2.5±1.2; median 3 [1–3]
Acetowhite present: No	118/268 (44.0%)	115/207 (55.6%)	3/61 (4.9%)
Acetowhite present: Yes	150/268 (56.0%)	92/207 (44.4%)	58/61 (95.1%)
Acetowhite intensity: None	126/268 (47.0%)	120/207 (58.0%)	6/61 (9.8%)
Acetowhite intensity: Mild	77/268 (28.7%)	62/207 (30.0%)	15/61 (24.6%)
Acetowhite intensity: Moderate	42/268 (15.7%)	19/207 (9.2%)	23/61 (37.7%)
Acetowhite intensity: Severe	23/268 (8.6%)	6/207 (2.9%)	17/61 (27.9%)
Punctuation: No	229/268 (85.4%)	195/207 (94.2%)	34/61 (55.7%)
Punctuation: Yes	39/268 (14.6%)	12/207 (5.8%)	27/61 (44.3%)
Atypical vessels: No	255/268 (95.1%)	202/207 (97.6%)	53/61 (86.9%)
Atypical vessels: Yes	13/268 (4.9%)	5/207 (2.4%)	8/61 (13.1%)

*Nine patients (5 in CIN2– group, 4 in CIN2+ group) had unknown or missing data for Multiple HR-HPV infection status

Table 2: Study Flow and Analysis Populations, Including Reference Verification Pathways

Item	N
Total records	300
Procedure-complete (target + 4Q performed)	294
ECC-eligible (prespecified criteria met)	149
Reference-standard evaluable (ref_available=1)	268
├ Excision histology (LEEP/conization)	128
└ 12-month follow-up verification	140
Paired comparative set (A and B2 available + ref)	268
Reference CIN2+ prevalence (among ref-evaluable)	61/268 (22.8%)
Reference CIN3+ prevalence (among ref-evaluable)	31/268 (11.6%)

Table 3: Diagnostic Performance of Strategies A, B1, and B2 For Reference Confirmed CIN2 Plus In The Reference Standard Evaluable Cohort (n=268). Values Are Counts and Proportions With 95 Percent Confidence Intervals

Strategy	n	TP	FP	TN	FN	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
A	268	46	1	206	15	75.4 (63.3–84.5)	99.5 (97.3–99.9)	97.9 (88.9–99.6)	93.2 (89.1–95.8)
B1	268	56	1	206	5	91.8 (82.2–96.4)	99.5 (97.3–99.9)	98.2 (90.7–99.7)	97.6 (94.6–99.0)
B2	268	58	1	206	3	95.1 (86.5–98.3)	99.5 (97.3–99.9)	98.3 (91.0–99.7)	98.6 (95.9–99.5)

Table 4. Incremental Yield and Number Needed To Sample For Systematic Four Quadrant Biopsies and Endocervical Curettage Among Reference CIN2 Plus Patients (n=61)

Component	Incremental CIN2+ (ref-confirmed)	Patient-based NNS	Specimen-based NNS
4-quadrant biopsies (B1 vs A)	10/61 (16.4%)	26.2	112.2
ECC (B2 vs B1)	2/61 (3.3%)	67.0	75.5
Total (B2 vs A)	12/61 (19.7%)		

Table 4: (continued). Sampling Burden and Operational Overdetection Proxies Among Reference CIN2 Minus Patients Negative Under Both Strategy A and Strategy B2 (n=206)

Overdetection / burden proxy	n/N (%)
Ref CIN2- & A- & B2- (denominator)	206/206 (100.0%)
Any CIN1 detected in additional 4Q biopsies	129/206 (62.6%)
Any CIN1 detected in ECC	9/206 (4.4%)
Any CIN1 detected in 4Q or ECC	135/206 (65.5%)
Extra sampling performed (4Q and/or ECC)	203/206 (98.5%)

Table 4: (continued). Net Incremental CIN2 Plus Gain Per Additional Specimen In The Reference Standard Evaluable Cohort (n=268)

Net gain per sampling	Value
Total additional specimens vs Strategy A (ref-evaluable cohort)	1298
Incremental CIN2+ captured (A-,B2+,ref+)	12
Incremental CIN2+ per additional specimen	0.92%

Table 5. Key Multivariable and Subgroup Summaries. Panel A Shows Selected Predictors of Reference CIN2 Plus (Model 1)

Predictor	OR (95% CI)	p- value
C(cytology_group)[T.Low-grade/Negative]	0.29 (0.11–0.75)	0.010
C(hpv_group)[T.HR-HPV Negative]	0.19 (0.04–0.99)	0.048
C(hpv_group)[T.HR-HPV Positive (type unk)]	0.45 (0.21–0.96)	0.039
C(tz_group)[T.TZ3]	1.22 (0.51–2.92)	0.659
age_years	1.02 (0.95–1.09)	0.656
acetowhite_present	20.66 (10.29–41.48)	<0.001

Table 5: (Continued). Panel B Shows Exploratory Predictors of Incremental Capture (Model 2) Among Reference CIN2 Plus Patients (n=61; events=12)

Predictor	OR (95% CI)	P -value
hpv16_18	2.11 (0.80–4.76)	0.150
cytology_highgrade	0.96 (0.39–2.06)	0.850
tz3	1.98 (0.79–4.56)	0.150
scj_not_full	0.39 (0.26–0.59)	<0.001
age_gt40	1.74 (0.68–3.95)	0.250
immunosuppression_yesno	0.70 (0.49–1.00)	0.050

Table 5: (Continued). Panel C Shows Prespecified Subgroup Incremental Yield For Strategy B2 Versus Strategy A Among Reference CIN2 Plus Patients

Subgroup	Level	n (ref+)	ΔSe	Incremental ΔSe
HPV16/18	HPV16/18	19	31.6%	31.6%
HPV16/18	No 16/18	42	14.3%	14.3%
Cytology grade	High-grade	43	20.9%	20.9%
Cytology grade	Low/Other	18	16.7%	16.7%
TZ type	TZ1-2	47	14.9%	14.9%
TZ type	TZ3	14	35.7%	35.7%
SCJ visibility	Partial/Not	61	19.7%	19.7%
Colposcopic impression	Normal/Minor	61	19.7%	19.7%
Age group	<30	12	16.7%	16.7%
Age group	30-39	29	10.3%	10.3%
Age group	40+	20	35.0%	35.0%

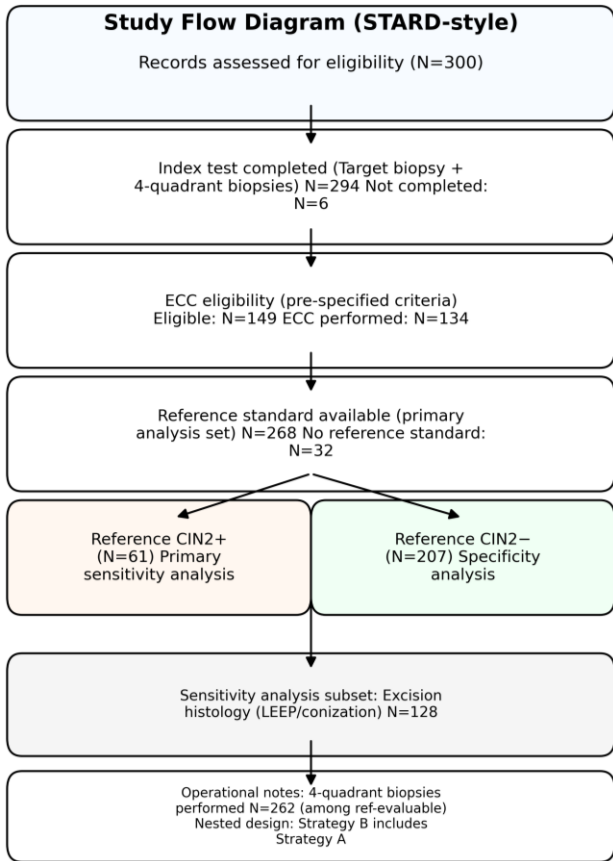


Fig. 2. Paired CIN2 plus detection within reference positive patients (n=61). Bars indicate the number of CIN2 plus cases detected by Strategy A, additional cases detected by adding systematic four quadrant biopsies, additional cases detected by adding endocervical curettage, and residual missed cases under each strategy

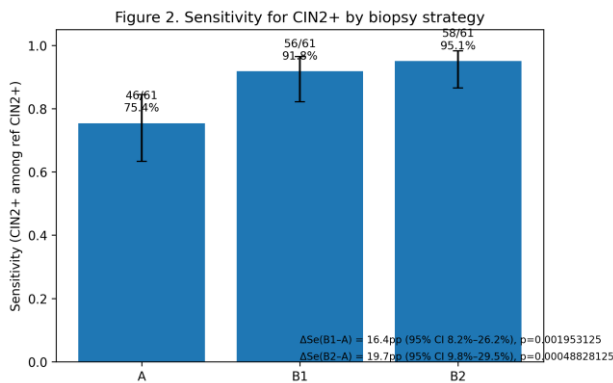


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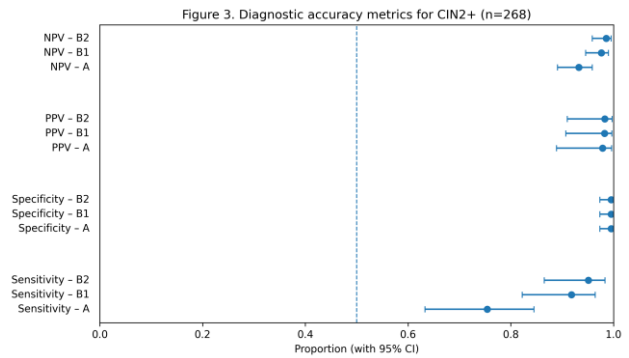


Fig. 3. Diagnostic accuracy metrics for CIN2 plus in the reference standard evaluable cohort (n=268). Points indicate estimates and horizontal lines indicate 95 percent confidence intervals for sensitivity, specificity, positive predictive value, and negative predictive value across Strategies A, B1, and B2

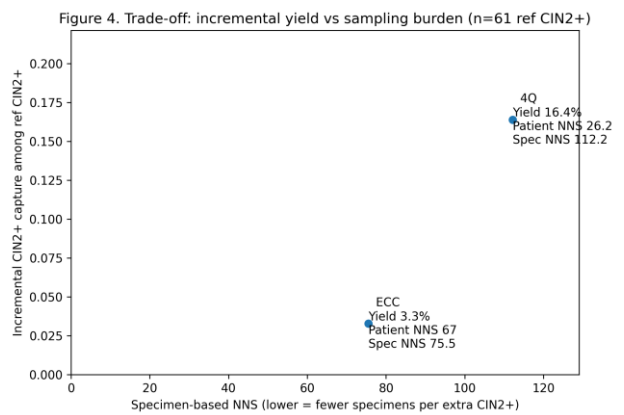


Fig. 4. Trade off between incremental CIN2 plus yield and sampling burden among reference CIN2 plus patients (n=61). The plot contrasts four quadrant biopsies and endocervical curettage using incremental yield and specimen based number needed to sample, with patient based number needed to sample shown for context

reference CIN2 minus patients who were negative under both Strategy A and Strategy B2 (n=206), CIN1 was detected in additional four quadrant biopsies in 62.6 percent and in endocervical curettage in 4.4 percent. Across the reference standard evaluable cohort, there were 1298 additional specimens beyond the primary targeted biopsy, corresponding to an incremental gain of 0.92 percent per additional specimen, or approximately one additional CIN2 plus detected per 108 additional specimens.

Prespecified Subgroups and Sensitivity Analysis: Prespecified subgroup summaries are shown in Table 5 and Figure 5. The largest incremental yields for Strategy B2 versus Strategy A were observed in transformation zone type 3, age 40 years or older, and HPV16 or HPV18

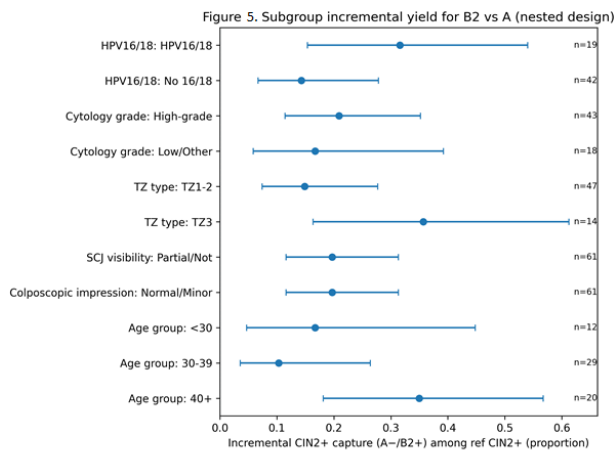


Fig. 5. Prespecified subgroup incremental yield for Strategy B2 versus Strategy A among reference CIN2 plus patients. Incremental yield corresponds to the proportion of reference CIN2 plus cases missed by Strategy A but detected by Strategy B2 within each subgroup

positive strata. In sensitivity analysis restricted to excision verified patients, the sensitivity advantage of strategies that included systematic sampling persisted (Supplementary analysis available on request).

Discussion

This paired diagnostic accuracy study showed that adding systematic four quadrant biopsies to a prespecified primary targeted biopsy substantially increased detection of reference confirmed CIN2 plus. Compared with Strategy A, Strategy B1 increased sensitivity by 16.4 percentage points, and Strategy B2 increased sensitivity by 19.7 percentage points. The main gain was attributable to four quadrant sampling, while endocervical curettage provided a smaller incremental yield that was not statistically significant in the overall reference positive subset.

These findings are consistent with evidence that multiple biopsies improve detection at colposcopy. Wentzensen and colleagues demonstrated that taking multiple biopsies increases detection of cervical precursors compared with fewer biopsies. (5) ASCCP colposcopy standards emphasize systematic assessment of the transformation zone and squamocolumnar junction and encourage practice standardization and quality improvement, which supports structured sampling strategies that reduce missed disease. (6-11) Our results also align with guidance on endocervical curettage that

supports selective use in higher risk profiles and settings of incomplete visualization. (12)

The clinical relevance of improved detection must be balanced against sampling burden and downstream harms. In our dataset, operational burden proxies showed frequent CIN1 detection in additional samples among reference negative patients, reflecting an increase in low grade findings that may not change management. Such findings should not be labelled as overdiagnosis in the strict epidemiologic sense, but they are measurable indicators of additional procedures and follow up burden. This balance is important because CIN2 can regress under surveillance in a substantial proportion of patients, and current evidence and consensus statements support active surveillance in selected populations. (18-20) Downstream treatment also matters, as excisional procedures are associated with adverse obstetric outcomes that increase with greater cone depth. (21) Broader position statements emphasize optimizing detection of clinically meaningful disease while limiting unnecessary procedures. (22)

This study has strengths that support interpretability. The paired within patient design with nested strategies directly quantifies incremental yield using discordant pairs and avoids confounding by case mix. Separating B1 and B2 clarifies the specific contribution of endocervical curettage. The presence of two reference verification pathways reflects real world practice, and the excision only analysis supports robustness to differential verification in this dataset.

Limitations include the composite reference standard, which can introduce differential verification bias, and the single center setting, which may limit generalizability. Subgroup and exploratory model findings should be interpreted as hypothesis generating and should be validated in prospective cohorts with standardized verification pathways.

In this paired evaluation, adding systematic four quadrant biopsies to targeted biopsy increased detection of reference confirmed CIN2 plus without an observed specificity penalty in this dataset. The incremental benefit was driven primarily by four quadrant sampling. Endocervical curettage added a smaller incremental yield overall and may be best reserved for selected higher risk profiles and settings of limited visualization. Detection gains should be interpreted alongside sampling burden and low grade findings in additional specimens.

Data Availability Statement: Deidentified data underlying the results reported in this article are available from the corresponding author upon reasonable request, subject to institutional policies and applicable regulations.

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References

1. US Preventive Services Task Force, Curry SJ, Krist AH, Owens DK, et al. Screening for cervical cancer: US Preventive Services Task Force recommendation statement. *JAMA*. 2018;320(7):674-686.
2. Fontham ETH, Wolf AMD, Church TR, et al. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. *CA Cancer J Clin*. 2020;70(5):321-346.
3. Brisson M, Kim JJ, Canfell K, et al. Impact of HPV vaccination and cervical screening on cervical cancer elimination: comparative modelling analysis in 78 low income and lower middle income countries. *Lancet*. 2020;395(10224):575-590.
4. Qin D, Bai A, Xue P, et al. Colposcopic accuracy in diagnosing squamous intraepithelial lesions: a systematic review and meta analysis of the International Federation of Cervical Pathology and Colposcopy 2011 terminology. *BMC Cancer*. 2023;23:187.
5. Wentzensen N, Walker JL, Gold MA, et al. Multiple biopsies and detection of cervical cancer precursors at colposcopy. *J Clin Oncol*. 2015;33(1):83-89.
6. Wright TC Jr. The new ASCCP colposcopy standards. *J Low Genit Tract Dis*. 2017;21(4):215.
7. Wentzensen N, Massad LS, Mayeaux EJ Jr, et al. Evidence based consensus recommendations for colposcopy practice for cervical cancer prevention in the United States. *J Low Genit Tract Dis*. 2017;21(4):216-222.
8. Khan MJ, Werner CL, Darragh TM, et al. ASCCP colposcopy standards: role of colposcopy, benefits, potential harms, and terminology for colposcopic practice. *J Low Genit Tract Dis*. 2017;21(4):223-229.
9. Wentzensen N, Schiffman M, Silver MI, et al. ASCCP colposcopy standards: risk based colposcopy practice. *J Low Genit Tract Dis*. 2017;21(4):230-234.
10. Waxman AG, Conageski C, Silver MI, et al. ASCCP colposcopy standards: how do we perform colposcopy? Implications for establishing standards. *J Low Genit Tract Dis*. 2017;21(4):235-241.
11. Mayeaux EJ Jr, Novetsky AP, Chelmow D, et al. ASCCP colposcopy standards: colposcopy quality improvement recommendations for the United States. *J Low Genit Tract Dis*. 2017;21(4):242-248.
12. Massad LS, Perkins RB, Naresh A, et al. Guidelines for endocervical curettage at colposcopy. *J Low Genit Tract Dis*. 2023. PMID: 36222824.
13. Perkins RB, Guido RS, Castle PE, 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(2):102-131.
14. Cheung LC, Egemen D, Chen X, et al. 2019 ASCCP risk based management consensus guidelines: methods for risk estimation, recommended management, and validation. *J Low Genit Tract Dis*. 2020;24(2):90-101.
15. Egemen D, Cheung LC, Chen X, et al. Risk estimates supporting the 2019 ASCCP risk based management consensus guidelines. *J Low Genit Tract Dis*. 2020;24(2):132-143.
16. Demarco M, Egemen D, Raine Bennett TR, et al. A study of partial human papillomavirus genotyping in support of the 2019 ASCCP risk based management consensus guidelines. *J Low Genit Tract Dis*. 2020;24(2):144-147.
17. Wentzensen N, Walker J, Smith K, et al. A prospective study of risk based colposcopy demonstrates improved detection of cervical precancers. *Am J Obstet Gynecol*. 2018;218(6):604.e1-604.e8.
18. Tainio K, Athanasiou A, Tikkinen KAO, et al. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta analysis. *BMJ*. 2018;360:k499.
19. Lycke KD, Kahlert J, Damgaard RK, et al. Clinical course of cervical intraepithelial neoplasia grade 2: a population based cohort study. *Am J Obstet Gynecol*. 2023;229:656.e1-656.e15.
20. Kyrgiou M, Athanasiou A, Paraskeva M, et al. Adverse obstetric outcomes after local

- treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta analysis. *BMJ*. 2016;354:i3633.
21. Kyrgiou M, Arbyn M, Bergeron C, et al. Cervical screening: ESGO EFC position paper of the European Society of Gynaecologic Oncology and the European Federation of Colposcopy. *Br J Cancer*. 2020;123:510-517.
 22. Kyrgiou M, Bowden SJ, Ellis LB, et al. Active surveillance of cervical intraepithelial neoplasia grade 2: BSCCP and ESGO consensus statement. *Lancet Oncol*. 2025;26:e140-e151.
 23. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ*. 2015;351:h5527.