

GYNECOLOGY

The role of misclassification in progression of cervical intraepithelial neoplasia grade 2 during active surveillance: results from a historical cohort



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BACKGROUND: Many countries have recently implemented active surveillance for cervical intraepithelial neoplasia grade 2 as an option in younger women due to high regression rates and because excisional treatment is associated with an increased risk of preterm birth. However, the known low reproducibility of the cervical intraepithelial neoplasia grade 2 diagnosis poses a major challenge for active surveillance, suggesting that the observed risk of progression in prior studies may be due to initial misclassification of the cervical intraepithelial neoplasia grade 2 diagnosis.

OBJECTIVE: This study aimed to assess the association between expert-verified histological diagnoses of cervical biopsies in women undergoing active surveillance for cervical intraepithelial neoplasia grade 2 and the subsequent risk of cervical intraepithelial neoplasia grade 3 or worse.

STUDY DESIGN: We conducted a historical cohort study on women undergoing active surveillance for cervical intraepithelial neoplasia grade 2, diagnosed at Aarhus University Hospital from 2000 to 2010. Women were identified through the Danish Pathology Data Bank and were eligible for inclusion if they were aged 23 to 40 years at the time of cervical intraepithelial neoplasia grade 2 diagnosis. Women with a prior history of cervical intraepithelial neoplasia grade 2 or worse, loop electrosurgical excision procedure, or hysterectomy were excluded. Three expert pathologists independently graded cervical lesions by reviewing hematoxylin and eosin and p16—stained tissue slides from the time of cervical intraepithelial neoplasia grade 2 diagnosis. Women were followed for 28 months, from the date of the cervical intraepithelial neoplasia grade 2 diagnosis until a record of progression, loop electrosurgical excision procedure, hysterectomy, or end of follow-up, whichever occurred first. Using modified Poisson regression analysis, we estimated the crude and adjusted relative risks of cervical intraepithelial neoplasia grade 3 or worse, including corresponding 95% confidence intervals. We adjusted for age, index cytology, and human papillomavirus genotype as potential confounders.

RESULTS: A total of 437 women were included with a median age of 27 years. Upon expert review, 56 (12.8%) were upgraded to cervical

intraepithelial neoplasia grade 3, 261 (59.7%) had cervical intraepithelial neoplasia grade 2 confirmed, and 120 (27.5%) were downgraded to cervical intraepithelial neoplasia grade 1/normal. Overall, 173 (39.6%) women had a subsequent record of cervical intraepithelial neoplasia grade 3 or worse, ranging from 22.5% in women with expert cervical intraepithelial neoplasia grade 1/normal, to 42.2% in women with expert cervical intraepithelial neoplasia grade 2, and 64.3% in women with expert cervical intraepithelial neoplasia grade 3. Thus, women with an expert-verified cervical intraepithelial neoplasia grade 3 diagnosis were more likely to have a subsequent record of cervical intraepithelial neoplasia grade 3 or worse during follow-up (adjusted relative risk, 1.37 [1.07; 1.75]) compared to those who had expert cervical intraepithelial neoplasia grade 2. This association remained significant in stratified analyses for women aged 31 to 40 years (adjusted relative risk, 1.85 [1.25; 2.76]), in women with high-grade index cytology (adjusted relative risk, 1.43 [1.04; 1.97]), and women with a nonhuman papillomavirus16 lesion (adjusted relative risk, 1.66 [1.09; 2.52]). The risk of cervical intraepithelial neoplasia grade 3 or worse remained high for women presenting with a high-grade cytology or human papillomavirus 16 positivity at baseline, irrespective of expert diagnosis.

CONCLUSION: Our findings demonstrate substantial interobserver variability in the diagnosis of community-detected cervical intraepithelial neoplasia grade 2, with nearly half of the cases being reclassified upon expert review. This underscores the heterogeneity of this diagnosis and the importance of accurate diagnosis to minimize both undertreatment and overtreatment. These results highlight the need for individualized, risk-based management strategies for cervical intraepithelial neoplasia grade 2 that integrate virological and cytological factors rather than relying solely on histopathology.

Key words: cervical intraepithelial neoplasia grade 2, clinical management, histopathology, interobserver variability, loop electrosurgical excision procedure, progression, risk assessment

Introduction

For many years, loop electrosurgical excision procedure (LEEP) has been the standard treatment for cervical intraepithelial neoplasia grade 2 (CIN2).

However, spontaneous regression rates for CIN2 are high (50%–63%),^{1–3} suggesting a significant risk of overtreatment if all women with CIN2 undergo LEEP.² Unnecessary LEEP should be avoided as they are associated with an increased risk of preterm birth in subsequent pregnancies.^{4,5} Consequently, many countries, including the United States, have implemented active surveillance for CIN2 (ie, leaving the

CIN2 lesion untreated) as an option in younger women.^{6–10} In Denmark, active surveillance for CIN2 was implemented in the Central Denmark Region in 1995 and nationwide in 2013,¹¹ creating a unique opportunity to investigate risk factors for progression to cervical intraepithelial neoplasia grade 3 or worse (CIN3+) in this group of women. Accurate identification of women at the highest risk of progression

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AJOG at a glance

Why was this study conducted?

Several countries have adopted active surveillance for cervical intraepithelial neoplasia grade 2 (CIN2) as an option in younger women due to high regression rates. However, the known low reproducibility of the CIN2 diagnosis poses a major challenge, suggesting that the risk of progression during the surveillance period may be due to initial misclassification of the CIN2 diagnosis.

Key findings

Upon expert review, 40.3% of the CIN2 diagnoses were misclassified. Women with an expert-verified cervical intraepithelial neoplasia grade 3 diagnosis were more likely to have a subsequent record of cervical intraepithelial neoplasia grade 3 or worse during follow-up. The risk of cervical intraepithelial neoplasia grade 3 or worse remained high for women with a high-grade cytology or human papillomavirus 16 positivity at baseline, irrespective of expert diagnosis.

What does this add to what is known?

Our results highlight the need for individualized, risk-based management strategies for CIN2 that integrate virological and cytological factors rather than relying solely on histopathology.

is essential to enable targeted and timely treatment. Consequently, several studies have investigated potential predictors of disease progression. Studies have shown that infection with human papillomavirus (HPV) 16, high-grade cytology at the time of CIN2 diagnosis, and lack of HPV vaccination are associated with an increased risk of progression.^{3,12,13} Nevertheless, the well-documented low reproducibility of the CIN2 diagnosis^{14–18} poses a major challenge for active surveillance. Building on our previous histopathological expert verification of a historical CIN2 cohort,¹⁹ this study aimed to assess the association between expert-verified histological diagnoses and subsequent risk of CIN3+ during the active surveillance period.

Materials and methods**Setting**

In Denmark, opportunistic cervical cancer screening began in the mid-1960s,²⁰ and the first guidelines were published in 1986, recommending triennial screening to women aged 23 to 59 years.²¹ Since 2007, women aged 23 to 64 years have been invited for screening every 3 to 5 years. HPV-based screening was implemented in 2012 for women aged 60 to 64 years, and in 2021,

it was gradually implemented for women aged 30 to 59 years. Screening, diagnostic work-up, treatment, and post-treatment follow-up are free of charge for all women.²² In case of an abnormal screening result, the woman may undergo repeat testing or be referred to a gynecologist for colposcopy and cervical punch biopsies, depending on the severity of the cytology results. Following biopsy, women with cervical intraepithelial neoplasia grade 1 (CIN1) undergo repeat testing after 6 to 12 months, whereas women with CIN3 or adenocarcinoma in situ are recommended a LEEP. In case of CIN2, women of reproductive age are offered active surveillance, while women beyond reproductive age are recommended immediate LEEP.²³ Active surveillance includes semiannual colposcopy with biopsies for up to 2 years, with LEEP being performed in case of progression or persistent CIN2.

Study design and population

We conducted a historical cohort study on nonpregnant women undergoing active surveillance for CIN2 as also described previously.¹⁹ Women were diagnosed at the Department of Pathology, Aarhus University Hospital,

Denmark, between January 1, 2000 and December 31, 2010. They were identified through the Danish Pathology Data Bank (DPDB) using Systematized Nomenclature in Medicine codes for moderate dysplasia (M74B09) and cervical biopsy (T83110). The DPDB was established in 1997 and stores information on all cytological and histological samples collected in Denmark at an individual level.²⁴

Women were eligible for inclusion if they were aged 23 to 40 years and had undergone active surveillance (ie, cervical biopsy at the first follow-up visit after CIN2 diagnosis). Women were excluded if they had a previous history of cervical intraepithelial neoplasia grade 2 or worse (CIN2+), LEEP, a hysterectomy, if biopsies were missing, or if the biopsy material was deemed inadequate for analysis.

From the DPDB, we collected information on clinical characteristics and results on all cervical cytology and histology specimens. The index cytology was defined as the most recent cervical cytology sample obtained within 6 months prior to the CIN2 diagnosis. Index cytology was categorized as $\leq$ low-grade (ie, normal cytology, atypical squamous cells of undetermined significance, or low-grade squamous interepithelial lesions) or high-grade (ie, high-grade interepithelial lesion, atypical glandular cells, atypical squamous cells—cannot exclude high-grade interepithelial lesion, or adenocarcinoma in situ).

Laboratory analyses

Archived formalin-fixed and paraffin-embedded cervical punch biopsies were retrieved from the Department of Pathology, Aarhus University Hospital, Denmark. Tissue specimens were sectioned for the purpose of another study described previously.²⁵ Sectioning, hematoxylin and eosin (H&E) staining, and p16 immunohistochemical staining were performed by experienced laboratory technicians at the Department of Pathology, Aarhus University Hospital.¹⁹ HPV genotyping was performed at Viroclinics-DDL, Rijswijk, The Netherlands¹⁹ using the

HPV SPF₁₀-DEIA-LiPA₂₅ system version 1 (Labo Biomedical Products, Rijswijk, The Netherlands).^{26,27} In the present study, we categorized women as HPV16 positive if they had HPV16 detected in the baseline CIN2 sample with or without any other HPV genotypes, while women with non-HPV16 included women who tested positive for other HPV genotypes than HPV16 or those who tested HPV negative.

Expert review

H&E and p16–stained slides were sent to an international expert panel for evaluation. The expert panel consisted of 3 pathologists, each with more than 20 years of experience in gynecological pathology. The review process included the evaluation of both H&E and p16–stained slides and was based on a study-specific protocol described elsewhere.¹⁹ The pathologists were blinded to information on age, index cytology, HPV result, and outcome. The expert CIN diagnosis was based on agreement between at least 2 pathologists.

Outcome

Our primary outcome was defined as a record of CIN3+ in the DPDB during the active surveillance period, while the secondary outcome was defined as a record of CIN2+. For each patient, we used the most severe diagnosis obtained from either biopsies or LEEP during the period. We chose a 28-month surveillance period to ensure inclusion of any histopathological diagnoses derived from LEEP performed due to persistence at the 2-year follow-up visit.

Statistical analyses

Baseline characteristics were reported descriptively using counts and proportions. A modified Poisson regression model with robust variances was used to estimate the relative risk (RR) of the outcomes, including corresponding 95% confidence intervals (CIs), using expert CIN2 as the reference group. Women contributed time at risk from the time of CIN2 diagnosis until the outcome, LEEP, hysterectomy, or end of follow-up, whichever occurred first. We adjusted for age, index cytology, and

HPV genotype. Estimates were reported overall and stratified by age group,^{23–40} calendar year (2000–2003, 2004–2007, and 2008–2010), index cytology ($\leq$ low-grade and high-grade cytology), and HPV genotype (HPV16 and non-HPV16). In compliance with Danish legislation, counts below 5 were not reported, and thus, due to a low case count, HPV-negative women were grouped with non-HPV16, while women with missing cytology were grouped with low-grade cytology. Data management was performed in Research Electronic Data Capture (Vanderbilt University, Nashville, TN; hosted by Aarhus University), and statistical analyses were performed using STATA version 17.0 (StataCorp, College Station, TX).

Ethics approval

The study was approved by the Danish Scientific Ethical Committee in Central Denmark Region (journal number 1-10-72-60-20) and registered at Aarhus University (2016-051-000001).²⁵

Results

A total of 3623 women with a community CIN2 diagnosis in the DPDB from 2000 to 2010 formed the source population. From this population, 1496 women were randomly selected for evaluation of eligibility to ensure equal representation across calendar years. Following this random selection, women were excluded based on predefined criteria from the DPDB (eg, age outside 23–40 years, prior CIN2+ diagnosis, prior LEEP or hysterectomy, or suspicion of CIN1 or CIN3 at community diagnosis), archival issues (eg, missing or inadequate tissue samples), or laboratory reasons (eg, tissue damage during recutting, missing blocks, or failed HPV genotyping). After all exclusion steps, 437 women included for analysis in the present study (Figure). These women had a median age of 27.1 years (interquartile range, 24.6–30.2). At baseline, 261 (59.7%) women had an expert-verified CIN2 diagnosis, whereas 56 (12.8%) women were upgraded to CIN3 and 120 (27.5%) were downgraded to CIN1 or normal (Table 1).

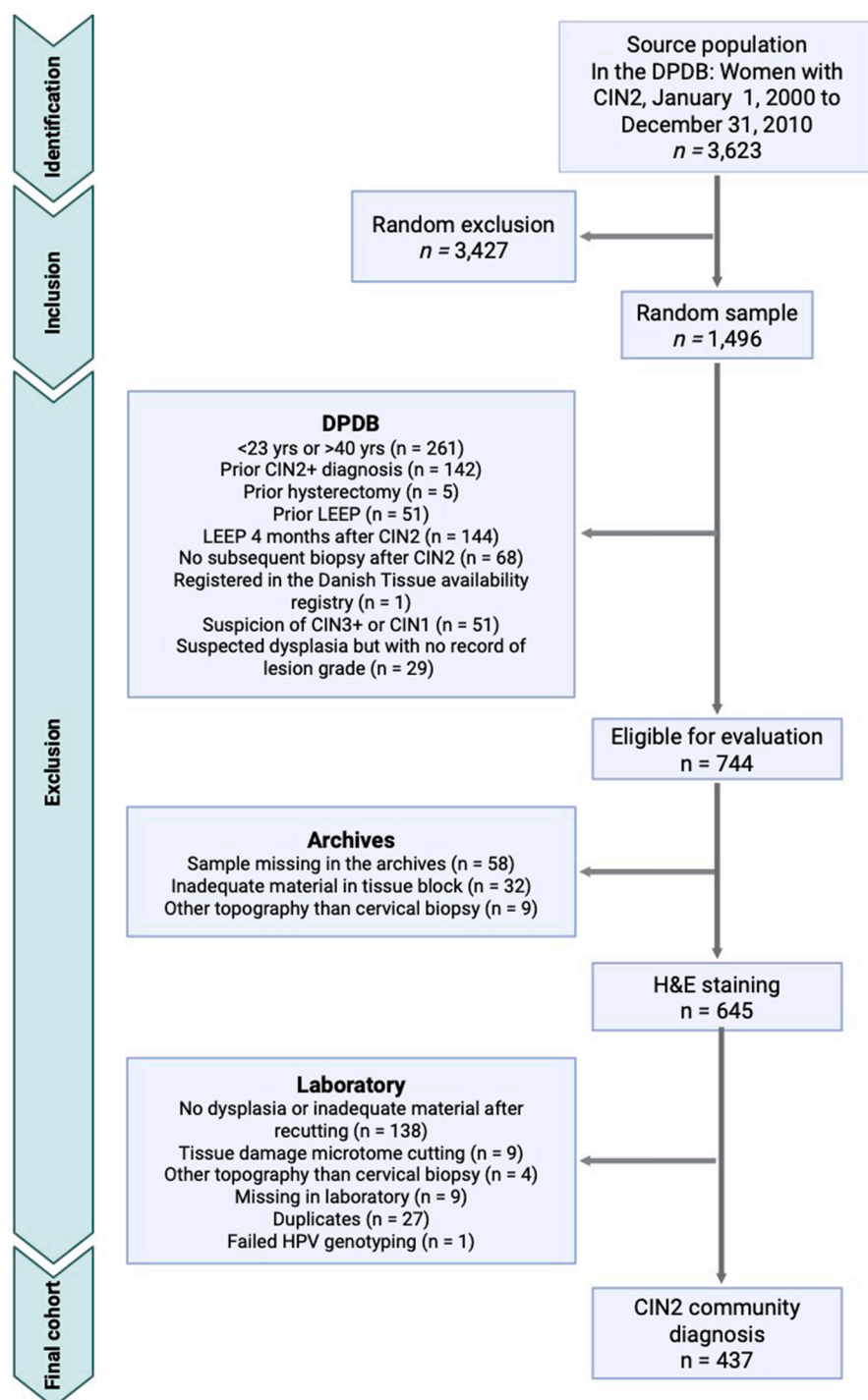
Importantly, no cancers were diagnosed following expert review of index biopsies. Compared to women who were downgraded to CIN1/normal, women with expert CIN2 and expert CIN3 were more likely diagnosed in the early time period, while no major trends were seen across groups with respect to age distribution or index cytology. HPV16 positivity increased with increasing severity, from 19.2% in women with expert CIN1/normal to 39.4% in expert CIN2, and 66.1% in expert CIN3.

Risk of cervical intraepithelial neoplasia grade 3 or worse

Overall, 173 women (39.6%) had a subsequent record of CIN3+ during follow-up (Table 2). Women with an expert CIN3 diagnosis were more likely to have a subsequent record of CIN3+ during follow-up (64.3%) compared to women with expert CIN1/normal (22.5%). Thus, compared to women with expert CIN2, women with expert CIN3 had a significantly higher risk of CIN3+ (adjusted relative risk [aRR], 1.37 [1.07; 1.75]), while women with expert CIN1/normal had a significantly lower risk of CIN3+ during follow-up (aRR, 0.62 [0.44; 0.88]) (Table 2).

Stratification by age at CIN2 diagnosis revealed interesting differences. In women aged 23 to 30 years, an expert CIN1/normal diagnosis was associated with a significantly lower risk of CIN3+ (aRR, 0.57 [0.37; 0.88]), while an expert CIN3 diagnosis was only slightly associated with an increased risk of CIN3+ (aRR, 1.23 [0.92; 1.65]). In contrast, for women aged 31 to 40 years, an expert CIN1/normal diagnosis was not associated with a lower risk of CIN3+ (aRR, 0.81 [0.45; 1.45]), while an expert CIN3 diagnosis was associated with 85% higher risk of CIN3+ (aRR, 1.85 [1.25; 2.76]) (Table 2).

In women with a low-grade index cytology, an expert CIN1/normal diagnosis was associated with a significantly lower risk of CIN3+ (aRR, 0.41 [0.21; 0.79]), while an expert CIN3 diagnosis trended toward increased risk, but this was not significant (aRR, 1.38 [0.94; 2.01]). For women with high-grade index cytology, those with expert CIN3

FIGURE
Selection flowchart

CIN2, cervical intraepithelial neoplasia grade 2; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; DPDB, Danish Pathology Data Bank; H&E, hematoxylin and eosin; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure.

(47.8%–67.6%) regardless of expert review (Table 2). Among non-HPV16–positive women, an expert CIN1/normal diagnosis was associated with a significantly lower risk of CIN3+ (aRR, 0.52 [0.32; 0.85]), while an expert CIN3 diagnosis was associated with a significantly increased risk of CIN3+ (aRR, 1.66 [1.09; 2.52]) (Table 2).

Risk of cervical intraepithelial neoplasia grade 2 or worse

Overall, 233 women (53.3%) had a record of CIN2+ during the surveillance period, ranging from 35% in women with expert CIN1/normal to 58.6% in women with expert CIN2, and 67.9% in women with expert CIN3 (Table 3). An expert CIN1/normal diagnosis was associated with a significantly lower risk of CIN2+ during follow-up (aRR, 0.67 [0.52; 0.87]), whereas women with expert CIN3 had a similar risk (aRR, 1.05 [0.86; 1.29]). Similar findings were observed in the youngest age group (23–30 years) and in women with low-grade cytology and non-HPV16–positive women, while we found no difference across histology groups in the oldest age group (31–40 years), in women with high-grade cytology, and in HPV16–positive women (Table 3).

Comment Principal findings

In this historical cohort study, we found that 27.5% of women with a community CIN2 diagnosis were downgraded to CIN1/normal, while 12.8% were upgraded to CIN3 by expert pathologists, thus indicating a significant risk of initial misclassification at CIN2 diagnosis. An expert CIN3 diagnosis was associated with a 64.3% increased risk of CIN3+ during follow-up; an association that remained significant in stratified analyses for women aged 31 to 40 years, women with high-grade cytology, and women with non-HPV16. Interestingly, we found a high absolute risk of progression in women with an associated high-grade cytology and those who tested positive for HPV16, irrespective of expert diagnosis.

had a significantly increased risk of CIN3+ during follow-up (aRR, 1.43 [1.04; 1.97]) (Table 2).

Stratification by HPV genotype showed that HPV16–positive women had a high absolute risk of CIN3+

TABLE 1

Baseline characteristics of women undergoing active surveillance for CIN2 in Denmark, 2000 to 2010

Patient characteristics	Total, N (%)	Expert CIN1/normal, n (%)	Expert CIN2, n (%)	Expert CIN3, n (%)
Total	437 (100)	120 (100)	261 (100)	56 (100)
Age at diagnosis				
23–30	347 (79.4)	99 (82.5)	203 (77.8)	45 (80.4)
31–40	90 (20.6)	21 (17.5)	58 (22.2)	11 (19.6)
Calendar year at diagnosis				
2000–2003	168 (38.4)	30 (25.0)	111 (42.5)	27 (48.2)
2004–2007	151 (35.6)	43 (35.8)	93 (35.6)	15 (26.8)
2008–2010	118 (27.0)	47 (39.2)	57 (21.9)	14 (25.0)
Index cytology				
≤ low-grade ^a	221 (50.6)	64 (53.3)	127 (48.7)	30 (53.6)
High-grade ^b	216 (49.4)	56 (46.7)	134 (51.3)	26 (46.4)
HPV genotype at diagnosis				
HPV16	163 (37.3)	23 (19.2)	103 (39.4)	37 (66.1)
Non-HPV16 ^c	274 (62.7)	97 (80.8)	158 (60.5)	19 (33.9)

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

^a ≤ low-grade cytology: Normal cytology, atypical squamous cells of undetermined significance (ASC-US), or low-grade squamous interepithelial lesions (LSILs); ^b High-grade cytology: High-grade interepithelial lesion (HSIL), atypical glandular cells (AGCs), atypical squamous cells—cannot exclude HSIL (ASC-H), or adenocarcinoma in situ (AIS); ^c Non-HPV16: HPV negative or HPV6, 11, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70, 74 positive.

Results in the context of what is known

In our study, community and expert pathologists agreed on 59.7% of the CIN2 diagnoses, which is a higher concordance compared to previous studies. In a prior study from the United States,²⁸ expert review of biopsies from 545 women diagnosed with CIN2 found agreement in only 43.4% of the CIN2 diagnoses. In contrast to our study, agreement on CIN grading was recorded if one community pathologist and one expert pathologist agreed, and with additional expert reviews in case of disagreement. They found that 29.3% of community CIN2 cases were downgraded to <CIN2 and 27.3% were upgraded to CIN3 by the experts, with no significant age differences between underdiagnosed and overdiagnosed women. In Stoler et al,¹⁸ agreement on CIN grading between community pathologists and 3 expert pathologists was made on 6272 biopsies from the New Mexico HPV Pap Registry database. Like in our study, the final CIN grade was based on agreement between at least 2 experts. Here, only 38% of the community-diagnosed CIN2 were

confirmed by the experts. Consistent with our findings, these studies underscore that CIN2 represents a heterogeneous group with substantial interobserver variability, thus emphasizing the critical importance of precise CIN grading to minimize the risk of undertreatment and overtreatment. Importantly, even with a relatively high rate of CIN2 misclassification at index, no cancers were identified in our cohort at baseline.

To reduce interobserver variability, our study identified age, cytology, and HPV genotype as important factors, while p16 staining has also shown its ability to guide pathologists toward better agreement.^{29,30} This was also shown in a previous study from our group, in which we found that p16-negative women with CIN2 were more likely to regress compared to p16-positive women.³¹ Importantly, expert pathologists in the present cohort were blinded to HPV results, and p16 immunostaining was not widely available in Denmark until the 2010s. Consequently, the limited availability of p16 at the time of CIN2 community diagnosis

is unlikely to have impacted our findings. If anything, misclassification may have been slightly underestimated, as cases assessed with p16 tend to be classified more accurately. Another critical factor that affects the CIN2 assessment but also the subsequent risk of progression and regression, however, is sampling. While a cytology sample is typically collected from the surface of the entire cervix, a biopsy is collected from one spot. Hence, the histopathological diagnosis is highly depended on the colposcopist's ability to identify and collect a biopsy from the worst lesion.^{32–34} The most effective way to minimize sampling error is to collect multiple biopsies. Studies have shown that taking a single biopsy targeting the worst appearing lesion may miss up to a third of precancers. Nevertheless, by collecting 2 targeted biopsies instead of one, the CIN2+ detection rate increases considerably from approximately 60% to 68% to 82% to 86%.^{35,36}

In our study, we had detailed information on the number and size of biopsies for the majority of women. The

TABLE 2

Absolute and relative risks of subsequent CIN3+ in women undergoing active surveillance, overall and stratified by age, cytology, and HPV genotype

Patient characteristics	Total, N	CIN3+ during follow-up, n (%)	Crude RR (95% CI)	Adjusted RR (95% CI) ^a
Overall				
Expert CIN1/normal	120	27 (22.5)	0.53 (0.37; 0.77)	0.62 (0.44; 0.88)
Expert CIN2	261	110 (42.2)	Ref.	Ref.
Expert CIN3	56	36 (64.3)	1.53 (1.20; 1.94)	1.37 (1.07; 1.75)
Age				
23–30				
Expert CIN1/normal	99	19 (19.2)	0.48 (0.31; 0.75)	0.57 (0.37; 0.88)
Expert CIN2	203	81 (39.9)	Ref.	Ref.
Expert CIN3	45	27 (60.0)	1.50 (1.12; 2.01)	1.23 (0.92; 1.65)
31–40				
Expert CIN1/normal	21	8 (38.1)	0.76 (0.42; 1.40)	0.81 (0.45; 1.45)
Expert CIN2	58	29 (50.0)	Ref.	Ref.
Expert CIN3	11	9 (81.8)	1.64 (1.12; 2.40)	1.85 (1.25; 2.76)
Index cytology				
≤ low-grade ^b				
Expert CIN1/normal	64	9 (14.1)	0.36 (0.19; 0.70)	0.41 (0.21; 0.79)
Expert CIN2	127	49 (38.6)	Ref.	Ref.
Expert CIN3	30	18 (60.0)	1.56 (1.08; 2.24)	1.38 (0.94; 2.01)
High-grade ^c				
Expert CIN1/normal	56	18 (32.1)	0.71 (0.46; 1.08)	0.81 (0.54; 1.21)
Expert CIN2	134	61 (45.5)	Ref.	Ref.
Expert CIN3	26	18 (69.2)	1.52 (1.11; 2.09)	1.43 (1.04; 1.97)
HPV genotype				
HPV16				
Expert CIN1/normal	23	11 (47.8)	0.86 (0.54; 1.37)	0.85 (0.54; 1.32)
Expert CIN2	103	57 (55.3)	Ref.	Ref.
Expert CIN3	37	25 (67.6)	1.22 (0.92; 1.62)	1.25 (0.94; 1.66)
Non-HPV16 ^d				
Expert CIN1/normal	97	16 (16.5)	0.49 (0.30; 0.81)	0.52 (0.32; 0.85)
Expert CIN2	158	53 (33.5)	Ref.	Ref.
Expert CIN3	19	11 (57.9)	1.73 (1.11; 2.69)	1.66 (1.09; 2.52)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; Ref., reference; RR, relative risk.

^a Adjusted for age, index cytology, and HPV genotype; ^b ≤ low-grade cytology: Normal cytology, atypical squamous cells of undetermined significance (ASC-US), or low-grade squamous intraepithelial lesions (LSILs); ^c High-grade cytology: High-grade intraepithelial lesion (HSIL), atypical glandular cells (AGCs), atypical squamous cells—cannot exclude HSIL (ASC-H) or adenocarcinoma in situ (AIS); ^d Non-HPV16: HPV negative or HPV6, 11, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70, 74 positive.

mean number of biopsies removed per woman was 2.5, with individual biopsies ranging from 2 to 8 mm, which is comparable to current clinical practice (3–5 mm). Consequently, the risk of

undersampling and missing the area with the highest grade of neoplasia is likely low.

In our study, we found a high absolute risk of CIN3+ in women positive for

HPV16, irrespective of expert diagnosis. In contrast, the risk varied considerably across expert histology groups in those who tested positive for non-HPV16. These findings demonstrate the

TABLE 3

Absolute and relative risks of CIN2+ in women undergoing active surveillance, overall and stratified by age, cytology, and HPV genotype

Patient characteristics	Total, N	CIN2+ during follow-up, n (%)	Crude RR (95% CI)	Adjusted RR (95% CI) ^a
Overall				
Expert CIN1/normal	120	42 (35.0)	0.60 (0.46; 0.78)	0.67 (0.52; 0.87)
Expert CIN2	261	153 (58.6)	Ref.	Ref.
Expert CIN3	56	38 (67.9)	1.16 (0.94; 1.42)	1.05 (0.86; 1.29)
Age				
23–30				
Expert CIN1/normal	99	32 (32.3)	0.57 (0.42; 0.78)	0.65 (0.48; 0.88)
Expert CIN2	203	115 (56.7)	Ref.	Ref.
Expert CIN3	45	28 (62.2)	1.10 (0.85; 1.42)	0.94 (0.73; 1.21)
31–40				
Expert CIN1/normal	21	10 (47.6)	0.73 (0.45; 1.18)	0.77 (0.49; 1.23)
Expert CIN2	58	38 (65.5)	Ref.	Ref.
Expert CIN3	11	10 (90.9)	1.39 (1.06; 1.81)	1.50 (1.11; 2.01)
Index cytology				
≤ low-grade ^b				
Expert CIN1/normal	64	15 (23.4)	0.43 (0.27; 0.69)	0.48 (0.29; 0.77)
Expert CIN2	127	69 (54.3)	Ref.	Ref.
Expert CIN3	30	20 (66.7)	1.23 (0.91; 1.66)	1.10 (0.81; 1.49)
High-grade ^c				
Expert CIN1/normal	56	27 (48.2)	0.77 (0.57; 1.04)	0.85 (0.64; 1.14)
Expert CIN2	134	84 (62.7)	Ref.	Ref.
Expert CIN3	26	18 (69.2)	1.10 (0.83; 1.47)	1.02 (0.77; 1.36)
HPV genotype				
HPV16				
Expert CIN1/normal	23	14 (60.9)	0.81 (0.58; 1.15)	0.80 (0.57; 1.12)
Expert CIN2	103	77 (74.8)	Ref.	Ref.
Expert CIN3	37	26 (70.3)	0.94 (0.74; 1.19)	0.96 (0.75; 1.22)
Non-HPV16 ^d				
Expert CIN1/normal	97	28 (28.9)	0.60 (0.42; 0.85)	0.62 (0.44; 0.89)
Expert CIN2	158	76 (48.1)	Ref.	Ref.
Expert CIN3	19	12 (63.2)	1.31 (0.90; 1.92)	1.28 (0.90; 1.82)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; Ref., reference; RR, relative risk.

^a Adjusted for age, index cytology, and HPV genotype; ^b ≤ low-grade cytology: Normal cytology, atypical squamous cells of undetermined significance (ASC-US), or low-grade squamous intraepithelial lesions (LSILs); ^c High-grade cytology: High-grade intraepithelial lesion (HSIL), atypical glandular cells (AGC), atypical squamous cells—cannot exclude HSIL (ASC-H), or adenocarcinoma in situ (AIS); ^d Non-HPV16: HPV negative or HPV6, 11, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70, 74 positive.

superior oncogenicity of HPV16 compared to other HPV genotypes.

Clinical implications

Our findings underscore the biological and clinical heterogeneity of CIN2 and highlight the importance of accurate

diagnostics, refined risk stratification, and shared decision-making when managing women with CIN2. A recent study on women diagnosed with CIN2 before pregnancy reported a 30% increased risk of preterm birth in women who had a delayed LEEP due to

persistent disease or progression during the active surveillance period compared to those who had a LEEP immediately after their CIN2 diagnosis.³⁷

These findings underscore the importance of identifying women with CIN2 who are at the highest risk of

progression who would benefit from immediate treatment. Although we found that the risk of CIN3+ during the surveillance period varied across expert diagnoses, progression risk depended on factors beyond histopathological assessment. In a large population-based cohort study,³ women undergoing active surveillance for CIN2 had a 58% increased risk of progression during active surveillance if they had an associated high-grade cytology compared to women with normal cytology. These findings may suggest that the cytology result reflects tumor biology and could serve as an independent prognostic marker as also indicated in the overall higher risk in women with an associated high-grade cytology, irrespective of expert diagnosis.

In our study, we found differences in the risk of progression by age. Thus, the absolute risk of CIN3+ was higher in women aged 31 to 40 years compared to women aged 23 to 30 years. These findings are in line with the conclusions of a systematic review, which reported lower risks in women below the age of 30 years,¹ while a prior Danish cohort study only reported a lower risk of progression in those aged 18 to 22 years.³ Interestingly, when stratifying by age, we found that an expert CIN1/normal diagnosis was associated with a lower risk of CIN3+ in women aged 23 to 30 years, while an expert CIN3 diagnosis was associated with a higher risk in women aged 31 to 40 years. Although the reason for this finding is unclear, it may be because older women have had the lesion for a longer time compared to younger women. Another important factor is HPV vaccination status as studies have shown that women vaccinated before age 15 have a lower risk of CIN3+ compared with unvaccinated women.^{13,38,39} Taken together, a multifactorial risk assessment based on histology, cytology, HPV genotype, and age should be considered when managing women with CIN2.

Research implications

Our study highlights the heterogeneity of the CIN2 diagnosis and the limitations of histology alone in predicting progression

risk. Biomarkers such as p16 immunostaining can substantially improve diagnostic accuracy, as p16 overexpression strongly correlates with transforming HPV infections and higher CIN grades as described in the Lower Anogenital Squamous Terminology guidelines.⁴⁰ Incorporating p16 into routine assessment has been shown to improve reproducibility of CIN2/CIN3 classification, which may improve risk stratification and guide more individualized management.^{29,30,41} Additional markers, including genomic features such as HPV integration status, lineage/sublineage, and specific single nucleotide variants, have been associated with patient prognosis and are being actively investigated.^{42–47} Emerging technologies, such as artificial intelligence–assisted histopathology, have shown promise in improving the consistency and accuracy of CIN2/CIN3 grading. For example, Andreassen et al⁴⁸ demonstrated that AI algorithms could detect CIN2+ lesions with high sensitivity and substantial agreement with expert pathologists, suggesting a potential role for AI in reducing interobserver variability and supporting more precise risk stratification. Future research should focus on integrating histology, cytology, HPV genotype, biomarker data, and artificial intelligence–assisted analysis to develop robust, individualized risk-stratification models.

Strengths and limitations

A strength of this study is its historical cohort design with complete follow-up, data collected from population-based registers, and the use of archived tissue specimens. All women undergoing active surveillance were eligible for inclusion without selection based on age, cytology, or other factors, thereby minimizing the risk of selection bias. The use of random sampling further reduced this risk. Another key strength is that each diagnosis was confirmed by consensus between 2 expert pathologists rather than a single expert reviewer.

A limitation of the study was the lack of detailed colposcopy data, including colposcopic impression, biopsy sites, and type of transformation zone. Furthermore, information on socioeconomic

status, ethnicity, smoking, and HPV vaccination status was not available. However, women included in the present study were not part of the vaccination program as the HPV vaccine was included in the Danish childhood vaccination program for girls at the age of 12 in 2009. While smoking may influence biological risk of progression, it does not affect the histopathological assessment itself. Thus, all women in this cohort had a histologically verified CIN2 diagnosis and were managed within the same surveillance framework. Smoking may still be associated with an increased risk of progression among women with CIN2, but such factors are less likely to be unevenly distributed across comparison groups than in general screening populations, where differences between participants and non-participants are more pronounced. Therefore, although residual confounding cannot be excluded, its impact is expected to be limited.

Given the study period of 2000 to 2010, women in our cohort were not part of the Danish childhood vaccination program, and therefore the prevalence of high-risk HPV types, particularly HPV16 and HPV18, is higher than in a vaccinated population.¹⁹ Consequently, with increasing HPV vaccination coverage, the overall incidence of CIN2 is expected to decrease, the genotype distribution will shift toward less carcinogenic types, and a higher proportion of lesions may persist or regress rather than progress. However, our study focuses on women who already have CIN2, and in this context, vaccination or population-level shifts in genotype prevalence are unlikely to alter the clinical course of these existing lesions. Overall, our findings should be interpreted in the context of a historical, unvaccinated cohort and may not fully reflect progression risks in women undergoing childhood HPV vaccination. Future studies are warranted to validate these observations in current, HPV-vaccinated populations.

For the statistical analyses, HPV-negative women were grouped with non-HPV16 cases, and women with missing index cytology were classified as low-grade cytology. While this

approach may have introduced some bias, the small number of affected cases suggests that any bias from misclassification would likely have reduced rather than increased the observed associations. We acknowledge that full HPV genotyping could provide additional risk-stratification insights. However, subgroup sizes were too small to allow a meaningful statistical analysis. Larger studies are therefore needed to fully explore genotype-specific risks.

Conclusion

Our results demonstrate the importance of standardized protocols for histopathological evaluation to ensure uniform diagnoses. Furthermore, our results highlight the need for refined risk stratification integrating clinical, histological, cytological, and virological parameters to improve clinical outcomes while reducing overtreatment.

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References

1. 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.
2. Loopik DL, Bentley HA, Eijgenraam MN, Int'Hout J, Bekkers RLM, Bentley JR. The natural history of cervical intraepithelial neoplasia grades 1, 2, and 3: a systematic review and meta-analysis. *J Low Genit Tract Dis* 2021;25:221–31.
3. 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.
4. Arbyn M, Kyrgiou M, Simoons C, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. *BMJ* 2008;337:a1284.
5. Kyrgiou M, Athanasiou A, Paraskevaidi 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.
6. 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;70:321–46.
7. Gov. UK. 2025. <https://www.gov.uk/government/publications/cervical-screening-programme-and-colposcopy-management/3-colposcopic-diagnosis-treatment-and-follow-up>. Accessed March 31, 2026.
8. Helsedirektoratet. 2025. <https://www.helse-direktoratet.no/retningslinjer/gynekologisk-kr-eft-handlingsprogram/premaligne-lidelser-i-cervix-uteri/innledning>. Accessed March 31, 2026.
9. Kunskapsbanken. <https://kunskapsbanken.cancercentrum.se/diagnoser/livmoderhalscan-cerprevention/wardprogram/handlaggning-ef-ter-utredning-som-visar-dysplasi/>. Accessed March 31, 2026.
10. Current care guidelines. 2023. <https://www.kaypahoito.fi/hoi50049>. Accessed March 31, 2026.
11. Lycke KD, Kahlert J, Petersen LK, et al. Untreated cervical intraepithelial neoplasia grade 2 and subsequent risk of cervical cancer: population based cohort study. *BMJ* 2023;383:e075925.
12. Damgaard RK, Jenkins D, Stoler MH, et al. Human papillomavirus genotypes and risk of persistence and progression in women undergoing active surveillance for cervical intraepithelial neoplasia grade 2. *Am J Obstet Gynecol* 2024;230:655.e1.
13. Krog L, Lycke KD, Kahlert J, et al. Risk of progression of cervical intraepithelial neoplasia grade 2 in human papillomavirus-vaccinated and unvaccinated women: a population-based cohort study. *Am J Obstet Gynecol* 2024;230:430.e1.
14. Carreon JD, Sherman ME, Guillen D, et al. CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. *Int J Gynecol Pathol* 2007;26:441–6.
15. Stoler MH, Schiffman M. Atypical squamous cells of undetermined significance-low-grade squamous intraepithelial lesion triage study G. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. *JAMA* 2001;285:1500–5.
16. Dalla Palma P, Giorgi Rossi P, Collina G, et al. The reproducibility of CIN diagnoses among different pathologists: data from histology reviews from a multicenter randomized study. *Am J Clin Pathol* 2009;132:125–32.
17. Ismail SM, Colclough AB, Dinnen JS, et al. Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia. *BMJ* 1989;298:707–10.
18. Stoler MH, Ronnett BM, Joste NE, et al. The interpretive variability of cervical biopsies and its relationship to HPV status. *Am J Surg Pathol* 2015;39:729–36.
19. Damgaard RK, Jenkins D, Stoler MH, et al. High prevalence of HPV16 and high-grade cytology in women undergoing active surveillance for cervical intraepithelial neoplasia grade 2. *Acta Obstet Gynecol Scand* 2023;102:1227–35.
20. Bigaard J, Hariri J, Lynge E. Cervical cancer screening in Denmark. *Eur J Cancer* 2000;36:2198–204.
21. Lynge E, Clausen LB, Guignard R, Poll P. What happens when organization of cervical cancer screening is delayed or stopped? *J Med Screen* 2006;13:41–6.
22. Screening for cervical cancer - recommendations. National Board of Health (Sundhedsstyrelsen), 2018 (in Danish). <https://www.sst.dk/da/udgivelser/2018/screening-for-livmoder-halskraeft>. Accessed March 31, 2026.
23. Guideline, Dansk selskab for gynækologi og obstetrik, 2013 (in Danish). <https://gynobsguide-line.dk/hindsgavl/Cervixdysplasi2012.pdf>. Accessed March 31, 2026.
24. Erichsen R, Lash TL, Hamilton-Dutoit SJ, Bjerregaard B, Vyberg M, Pedersen L. Existing data sources for clinical epidemiology: the Danish National Pathology Registry and Data Bank. *Clin Epidemiol* 2010;2:51–6.
25. Damgaard RK, Jenkins D, de Koning MN, et al. Performance of HPV E4 and p16(INK4a) biomarkers in predicting regression of cervical intraepithelial neoplasia grade 2 (CIN2): protocol for a historical cohort study. *BMJ Open* 2022;12:e059593.
26. Geraets DT, Struijk L, Kleter B, et al. The original SPF10 LIPA25 algorithm is more sensitive and suitable for epidemiologic HPV research than the SPF10 INNO-LiPA extra. *J Virol Methods* 2015;215-216:22–9.
27. van Alewijk D, Kleter B, Vent M, et al. A human papilloma virus testing algorithm comprising a combination of the L1 broad-spectrum SPF10 PCR assay and a novel E6 high-risk multiplex type-specific genotyping PCR assay. *J Clin Microbiol* 2013;51:1171–8.
28. Castle PE, Stoler MH, Solomon D, Schiffman M. The relationship of community biopsy-diagnosed cervical intraepithelial neoplasia grade 2 to the quality control pathology-reviewed diagnoses: an ALTS report. *Am J Clin Pathol* 2007;127:805–15.
29. Stoler MH, Wright TC Jr, Ferenczy A, et al. Routine use of adjunctive p16 immunohistochemistry improves diagnostic agreement of cervical biopsy interpretation: results from the CERTAIN Study. *Am J Surg Pathol* 2018;42:1001–9.
30. Reuschenbach M, Wentzensen N, Dijkstra MG, von Knebel Doeberitz M, Arbyn M. p16INK4a immunohistochemistry in cervical biopsy specimens: a systematic review and meta-analysis of the interobserver agreement. *Am J Clin Pathol* 2014;142:767–72.
31. Damgaard R, Jenkins D, Stoler MH, et al. p16(INK4a) and HPV E4 immunohistochemistry for the prediction of regression of cervical intraepithelial neoplasia grade 2-A historical cohort study. *Int J Cancer* 2025;157:1294–303.

32. Numnum TM, Kirby TO, Leath CA 3rd, Huh WK, Alvarez RD, Straughn JM Jr. A prospective evaluation of "see and treat" in women with HSIL Pap smear results: is this an appropriate strategy? *J Low Genit Tract Dis* 2005;9:2–6.
33. Barut MU, Kale A, Kuyumcuoglu U, et al. Analysis of sensitivity, specificity, and positive and negative predictive values of smear and colposcopy in diagnosis of premalignant and malignant cervical lesions. *Med Sci Monit* 2015;21:3860–7.
34. Fachetti-Machado G, Figueiredo-Alves RR, Moreira MAR. Performance of conventional cytology and colposcopy for the diagnosis of cervical squamous and glandular neoplasias. *Rev Bras Ginecol Obstet* 2018;40:410–6.
35. Gage JC, Hanson VW, Abbey K, et al. Number of cervical biopsies and sensitivity of colposcopy. *Obstet Gynecol* 2006;108:264–72.
36. Wentzensen N, Walker JL, Gold MA, et al. Multiple biopsies and detection of cervical cancer precursors at colposcopy. *J Clin Oncol* 2015;33:83–9.
37. Lycke KD, Kahlert J, Eriksen DO, et al. Preterm birth following active surveillance vs loop excision for cervical intraepithelial neoplasia grade 2. *JAMA Netw Open* 2024;7:e242309.
38. Baldur-Felskov B, Dehlendorff C, Munk C, Kjaer SK. Early impact of human papillomavirus vaccination on cervical neoplasia—nationwide follow-up of young Danish women. *J Natl Cancer Inst* 2014;106:djt460.
39. Eriksen DO, Jensen PT, Schroll JB, Hammer A. Human papillomavirus vaccination in women undergoing excisional treatment for cervical intraepithelial neoplasia and subsequent risk of recurrence: a systematic review and meta-analysis. *Acta Obstet Gynecol Scand* 2022;101:597–607.
40. Darragh TM, Colgan TJ, Cox JT, et al. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the college of American pathologists and the American Society for Colposcopy and Cervical Pathology. *J Low Genit Tract Dis* 2012;16:205–42.
41. Galgano MT, Castle PE, Atkins KA, Brix WK, Nassau SR, Stoler MH. Using biomarkers as objective standards in the diagnosis of cervical biopsies. *Am J Surg Pathol* 2010;34:1077–87.
42. Leeman A, Jenkins D, Del Pino M, et al. Expression of p16 and HPV E4 on biopsy samples and methylation of FAM19A4 and miR124-2 on cervical cytology samples in the classification of cervical squamous intraepithelial lesions. *Cancer Med* 2020;9:2454–61.
43. Clifford GM, Tenet V, Georges D, et al. Human papillomavirus 16 sub-lineage dispersal and cervical cancer risk worldwide: whole viral genome sequences from 7116 HPV16-positive women. *Papillomavirus Res* 2019;7:67–74.
44. Mirabello L, Yeager M, Yu K, et al. HPV16 E7 genetic conservation is critical to carcinogenesis. *Cell* 2017;170:1164–11674.e6.
45. Vinokurova S, Wentzensen N, Kraus I, et al. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. *Cancer Res* 2008;68:307–13.
46. Lang Kuhs KA, Faden DL, Chen L, et al. Genetic variation within the human papillomavirus type 16 genome is associated with oropharyngeal cancer prognosis. *Ann Oncol* 2022;33:638–48.
47. Oumeslakht L, Ababou M, Badaoui B, Qmichou Z. Worldwide genetic variations in high-risk human papillomaviruses capsid L1 gene and their impact on vaccine efficiency. *Gene* 2021;782:145533.
48. Andreassen AK, Mortensen E, Stenbro R, Sorensen O, Sorbye SW. Digital pathology with AI for cervical biopsies: diagnostic accuracy at the CIN2+ threshold. *Cancers (Basel)* 2025;17:3808.

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