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Post-Treatment Surveillance After Conisation for CIN2/3 in Norway: Long-Term CIN3+ Risk Stratification Comparing HPV DNA Testing with Cytology/HPV16/18/45 mRNA Co-Testing

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Abstract

We compared surveillance strategies in a retrospective regional cohort of 2136 women who underwent conisation for cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) in 2016–2020. Using results from the first eligible visit 90–548 days after treatment, we evaluated cytology, human papillomavirus (HPV) DNA testing and HPV16/18/45 E6/E7 messenger RNA (mRNA) testing, individually and in combination. We also evaluated an exploratory referral strategy adapted from the Addressing the Need for Advanced HPV Diagnostics (ATHENA) study. Histological outcomes were ascertained through 2025. Median available administrative follow-up was 7.24 years. CIN3+ (CIN3, adenocarcinoma in situ or invasive cervical cancer) was recorded in 74 women (3.5%). For CIN3+, HPV DNA testing had 93.2% sensitivity, 78.0% specificity and 24.4% positivity. Adding mRNA positivity to high-grade+ cytology increased CIN3+ sensitivity from 60.8% to 82.4%, with 92.1% specificity and 10.4% test positivity. Combining ASC-US+ cytology with mRNA positivity achieved the highest observed CIN3+ sensitivity (95.9%), with 73.5% specificity. The ATHENA-derived strategy achieved 90.5% sensitivity and 83.1% specificity, with 19.4% classified as referral-positive. Among women with equivocal or low-grade cytology, CIN3+ was recorded in 18.6% with positive mRNA and 2.5% with negative mRNA. Cytology and genotype-specific mRNA co-testing may support risk stratification after conisation. Prospective evaluation is needed to establish safe surveillance intervals.

Keywords: post-treatment surveillance; conisation; cervical intraepithelial neoplasia; HPV DNA testing; HPV E6/E7 mRNA; cervical cytology; co-testing; risk stratification; colposcopy; cervical cancer prevention

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1. Introduction

Persistent infection with oncogenic human papillomavirus (HPV) is the principal cause of cervical cancer and its precursor lesions [1]. Screening and treatment of high-grade cervical intraepithelial neoplasia (CIN) are central to cervical cancer prevention [2]. Women treated for CIN2/3 remain at increased risk of cervical precancer and cancer [3]. Post-treatment surveillance aims to detect high-grade disease while limiting unnecessary investigations [4].

HPV DNA testing is sensitive for residual or recurrent CIN2+ [4,5], and a negative result identifies women at low risk [4,5]. Some women remain HPV DNA-positive without

a subsequent diagnosis of high-grade disease, highlighting the need for further risk stratification [5]. Cytology provides complementary morphological information [6]. Equivocal or low-grade findings are interpreted alongside HPV results and treatment history [4].

HPV E6/E7 messenger RNA (mRNA) testing detects transcription of viral oncogenes [7]. PreTect® SEE detects E6/E7 mRNA from HPV16, HPV18 and HPV45 [8]. These genotypes account for most HPV-associated invasive cervical cancers and are particularly prevalent in HPV-associated adenocarcinoma [9]. Cancers associated with these genotypes tend to be diagnosed at younger ages [9,10]. Together with the biological relevance of E6/E7 oncogene expression, these characteristics support evaluating HPV16/18/45 E6/E7 mRNA detection as a complementary marker to cytology in post-treatment surveillance. Genotype-specific mRNA testing has been studied after excisional treatment [11,12]. Carcea et al. evaluated a five-genotype assay as a marker of treatment failure [11,12] and reported higher specificity for recurrent CIN2+ with mRNA than with HPV DNA testing (96.9% versus 82.8%) [12].

The implications of abnormal cytology depend on lesion severity and clinical history [4]. In routine practice, adding HPV16/18/45 mRNA results to cytology improved risk discrimination within cytological categories [8]. Whether this combination improves risk stratification after conisation, and how the choice of cytological threshold affects sensitivity and test positivity, remain unclear.

We studied 2136 women who underwent conisation for CIN2/3 in 2016–2020, with histological outcomes ascertained through 2025. Using results from the first eligible post-treatment visit, we compared cytology, 14-type HPV DNA testing (cobas 4800) and HPV16/18/45 mRNA testing (PreTect® SEE; PreTect AS, Klokkarstua, Norway), individually and in predefined combinations, for CIN2+ and CIN3+. We also evaluated an exploratory referral strategy adapted from the Addressing the Need for Advanced HPV Diagnostics (ATHENA) study [13], with referral indicated by HPV16 or HPV18 DNA positivity regardless of cytology, or by positivity for other oncogenic HPV types combined with ASC-US+ cytology. We assessed sensitivity, specificity and test positivity across strategies and examined whether mRNA status differentiated risk within cytological categories.

2. Materials and Methods

2.1. Study Design and Setting

This retrospective regional cohort study included women who underwent conisation for cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) in Troms and Finnmark, Northern Norway, during 2016–2020. All index conisations were performed using the loop electrosurgical excision procedure (LEEP). The Department of Clinical Pathology at the University Hospital of North Norway (UNN), which provides cervical pathology services for the region, examined all qualifying excision specimens.

Routine post-treatment management was guided by cervical cytology and HPV DNA results. HPV E6/E7 mRNA testing was performed as part of a local quality assurance project, and the results were used in this study to evaluate alternative surveillance strategies retrospectively. Results from the first eligible post-treatment visit were linked to histological diagnoses recorded at or after the index visit through 2025. Comparative analyses were restricted to women with valid results for all three testing modalities, as detailed in the eligibility criteria.

The study retrospectively evaluated alternative surveillance strategies using routinely collected clinical data. Women were not prospectively assigned to different surveillance pathways.

2.2. Data Source and Record Linkage

Data were retrieved from SymPathy (FlexLab/SymPathy v5.14.4.2; Tietoevry, Espoo, Finland), the laboratory information system and clinical pathology database used at UNN. SymPathy contains records of cervical cytology, HPV tests, cervical biopsies, cervical conisation specimens and hysterectomy specimens examined at the department. This enabled linkage of treatment specimens, post-treatment test results and subsequent histological diagnoses within the regional pathology service.

Individual patient records were linked using the national personal identification number. Relevant information included the date of conisation, post-treatment specimen collection dates, cytological diagnoses, HPV DNA and mRNA results, and histological diagnoses recorded during follow-up. Specimen collection dates were used to establish the temporal sequence of treatment, surveillance testing and histological outcomes.

2.3. Study Population and Index Post-Treatment Visit

The source population comprised 2625 women who underwent conisation for CIN2/3 during 2016–2020 and whose excision specimens were examined at UNN. For each woman, the first conisation during this period was defined as the index conisation.

Women were eligible for the index cohort if they had a cervical sample collected 90–548 days after conisation, analysed at UNN and yielding a valid HPV DNA result. Women without a sample meeting all three criteria were excluded from the index cohort. For each of the 2395 eligible women, the first sample meeting these criteria was selected as the index sample. The interval from conisation to the index visit was calculated using specimen collection dates. Samples analysed outside UNN were not systematically captured.

For the comparative analyses, women also needed evaluable cytology and a valid HPV16/18/45 E6/E7 mRNA result at the index visit. Of the 2395 women in the index cohort, 259 were excluded because of inadequate cytology, an invalid mRNA result or no linked mRNA result. The final analytical cohort comprised 2136 women, each contributing one index visit. The same cohort was used for all six testing strategies and the exploratory ATHENA-derived referral classification. Eligibility was based on index test results irrespective of subsequent histological outcomes.

2.4. Cervical Cytology

Cervical samples were collected in ThinPrep PreservCyt solution (Hologic, Marlborough, MA, USA) and processed for liquid-based cytology according to routine laboratory procedures. Cytological assessment was performed by cytotechnologists and pathologists at UNN, with diagnoses reported according to the Bethesda System for Reporting Cervical Cytology [14].

Two cytological positivity thresholds were evaluated. The ASC-US+ threshold included atypical squamous cells of undetermined significance (ASC-US) and all more severe squamous or glandular abnormalities. The high-grade+ threshold was operationally defined to include atypical glandular cells (AGUS or AGC), atypical squamous cells in which a high-grade squamous intraepithelial lesion could not be excluded (ASC-H), high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma in situ (AIS), or cytological malignancy. This operational category included glandular atypia irrespective of whether the cytological diagnosis itself established a high-grade lesion.

For analyses of risk stratification according to mRNA status, cytological findings were grouped into three mutually exclusive categories: negative for intraepithelial lesion or malignancy (NILM), ASC-US or low-grade squamous intraepithelial lesion (ASC-US/LSIL), and high-grade+ cytology.

2.5. HPV DNA Testing

HPV DNA testing was performed using the cobas 4800 HPV Test (Roche Molecular Systems, Pleasanton, CA, USA) according to the manufacturer's instructions and routine laboratory procedures. The assay detects 14 high-risk HPV genotypes, reporting HPV16 and HPV18 individually and providing a pooled result for 12 additional genotypes: HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68.

For the present analyses, HPV DNA positivity was defined as a positive result in at least one of the three assay channels. An HPV DNA-negative result required a valid assay result with no HPV detected in any channel.

2.6. HPV E6/E7 mRNA Testing

HPV E6/E7 mRNA testing was performed using PreTect® SEE (PreTect AS, Klokkarstua, Norway), a qualitative assay that detects E6/E7 mRNA from HPV16, HPV18 and HPV45 individually. The assay uses nucleic acid sequence-based amplification (NASBA) with genotype-specific detection and an intrinsic sample control to assess sample validity.

Testing was performed on residual liquid-based cervical specimens according to the manufacturer's instructions. During 2016–2020, UNN routinely performed PreTect® SEE testing on cervical cytology specimens, irrespective of the reason for sampling or the cytology and HPV DNA results. Testing was part of a local quality assurance project aimed at reducing the risk of cervical cancer after normal cytology [8]. After conisation, clinical management was guided by HPV DNA and cytology results. In this study, HPV mRNA results were used solely to evaluate alternative post-treatment surveillance strategies retrospectively. PreTect® SEE testing was discontinued in 2021, limiting the availability of mRNA results for women treated in 2020 whose first eligible post-treatment visit occurred in 2021.

An mRNA result was classified as positive if E6/E7 mRNA was detected from at least one of the three targeted genotypes and as negative if the result was valid and no E6/E7 mRNA was detected from any of them. Women with an invalid mRNA result or no linked mRNA record at the index visit were excluded from the common analytical cohort.

2.7. Surveillance Strategies

In routine care, HPV DNA and cytology results guided post-treatment management under the Norwegian national guidelines. HPV mRNA results were used in this study to evaluate alternative strategies retrospectively. Using results from the first eligible post-treatment visit, we applied six testing strategies to the same analytical cohort:

1. ASC-US+ cytology.
2. High-grade+ cytology.
3. HPV DNA positivity.
4. HPV16/18/45 E6/E7 mRNA positivity.
5. ASC-US+ cytology or HPV16/18/45 E6/E7 mRNA positivity.
6. High-grade+ cytology or HPV16/18/45 E6/E7 mRNA positivity.

In the two cytology/mRNA co-testing strategies, a result was classified as positive if cytology met the specified threshold, the mRNA result was positive, or both criteria were met. A negative classification required cytology below the specified threshold and a negative mRNA result.

We also evaluated an exploratory referral classification adapted from the ATHENA primary HPV screening strategy [13]. Women were assigned to four mutually exclusive groups:

1. HPV DNA-negative.

2. HPV16 and/or HPV18 DNA-positive, irrespective of cytology or concurrent positivity for other oncogenic HPV genotypes.
3. HPV DNA-positive exclusively for other oncogenic genotypes, with ASC-US+ cytology.
4. HPV DNA-positive exclusively for other oncogenic genotypes, with negative cytology (NILM).

Groups 2 and 3 were classified as referral-positive, and groups 1 and 4 as referral-negative. The referral-positive proportion was calculated by dividing the combined number of women in groups 2 and 3 by the total number of women in the analytical cohort.

Test positivity and referral positivity were calculated from the prespecified classification rules. Actual colposcopy and biopsy referrals were determined in routine care. The ATHENA-derived classification was evaluated as an exploratory comparator. Its use to extend surveillance intervals or return women to routine screening would require clinical validation.

2.8. Follow-Up and Histological Endpoints

Histological outcomes were ascertained from cervical biopsies, repeat conisation specimens and hysterectomy specimens recorded in SymPathy at or after the index visit through 31 December 2025. Histological diagnoses were established during routine clinical care. For analysis, recorded diagnoses were grouped as no dysplasia or malignancy, CIN1, CIN2, CIN3, adenocarcinoma in situ (AIS), or invasive cervical carcinoma. Squamous intraepithelial lesions were categorised as LSIL (CIN1) or HSIL (CIN2 or CIN3), consistent with WHO terminology, with CIN2 and CIN3 retained separately for endpoint definition [15]. Invasive carcinomas included squamous cell carcinoma and adenocarcinoma. The original conisation specimen was used to define the treatment episode and was not considered a follow-up endpoint.

Available administrative follow-up was defined as the interval between the index sample collection date and 31 December 2025. This interval represented the potential observation period determined by the study end date, irrespective of whether a histological endpoint was recorded earlier. It did not represent the duration of documented clinical surveillance or establish that each woman underwent continuous follow-up.

The study endpoints were histologically confirmed CIN2+, CIN3+ and invasive cervical cancer. CIN2+ included CIN2, CIN3, adenocarcinoma in situ (AIS) and invasive cervical cancer, whereas CIN3+ included CIN3, AIS and invasive cervical cancer. The endpoints were nested, with invasive cervical cancer included in both CIN2+ and CIN3+. Each woman was classified according to the most severe histological diagnosis recorded at or after the index visit through 31 December 2025. Endpoint classification and endpoint-specific dates were recorded separately. Time to registered CIN2+, CIN3+ and invasive cervical cancer was calculated from the index sample collection date to the corresponding histological specimen collection date, using separate date variables for each endpoint. The CIN3+ timing analysis used the CIN3+ date rather than the first CIN2+ date; these dates could differ when CIN2 was diagnosed before CIN3, AIS or invasive cancer.

Women without a registered endpoint were classified as having no recorded CIN2+ or CIN3+, as applicable. This category included women without histological sampling and women whose recorded histology did not meet the relevant endpoint definition. Accordingly, absence of a recorded endpoint did not necessarily indicate histologically verified absence of disease.

Histological assessment was based on haematoxylin and eosin-stained sections. In diagnostically equivocal squamous lesions, particularly those with a morphological differential diagnosis including CIN2, p16 immunohistochemistry was used at the reporting pathologist's discretion to support classification. Staining was interpreted in conjunction

with morphology, with strong, diffuse block-positive staining supporting a diagnosis of HSIL [16].

Histological verification was performed according to routine clinical indications rather than systematically in all participants. Consequently, the analyses describe associations between index test results and histologically confirmed disease recorded during the available observation period. Lesions identified at or after the index visit could represent residual, persistent or subsequently arising disease; these mechanisms were not distinguished in the endpoint definitions. The interval to diagnosis reflected the timing of histological ascertainment rather than the time of lesion development.

2.9. Statistical Analysis

The woman was the unit of analysis. Categorical variables were summarised as counts and percentages, and continuous variables as medians and interquartile ranges, with ranges where relevant. Women included in the analytical cohort and those excluded because of incomplete index testing were compared descriptively by age, calendar year, test availability and registered histological outcomes. Reasons for exclusion could overlap.

For each strategy, we reported the proportion classified as test-positive or, for the ATHENA-derived classification, referral-positive. Index classifications were cross-tabulated against registered CIN2+ and CIN3+ to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), with Wilson 95% confidence intervals (CIs). Exact two-sided McNemar tests compared paired sensitivities among women with the corresponding endpoint and paired specificities among women without it. Statistical significance was set at $p < 0.05$, with no adjustment for multiple comparisons. Non-significant paired comparisons were not interpreted as evidence of equivalence or non-inferiority.

Within each cytology category (NILM, ASC-US/LSIL and high-grade+), we estimated CIN2+ and CIN3+ proportions by mRNA status with Wilson 95% CIs. Risk ratios comparing mRNA-positive with mRNA-negative women had 95% CIs calculated on the log scale. Endpoint proportions were also summarised for the four ATHENA-derived groups and reflect diagnoses registered during the available follow-up.

Timing analyses were restricted to women with the corresponding endpoint and used the endpoint-specific specimen dates. We summarised intervals from the index visit and calculated the proportions of CIN2+ and CIN3+ endpoints recorded within 365 days and more than 730 days after that visit. The six invasive cancers were described using counts, proportions, Wilson 95% CIs where reported, and individual case characteristics and intervals.

Data management and analyses were performed using IBM SPSS Statistics version 32.0.0.0 (IBM Corp., Armonk, NY, USA) and Python version 3.12.13 (Python Software Foundation, Beaverton, OR, USA). Figures were generated in Python. All calculations used unrounded values.

3. Results

3.1. Study Population and Availability of Post-Treatment Test Results

A total of 2625 women underwent conisation during 2016–2020, of whom 2565 (97.7%) were represented in the available cytology/HPV dataset. An eligible index post-treatment sample, collected 90–548 days after conisation and with a valid HPV DNA result, was identified for 2395 women (91.2% of the baseline cohort). The median interval from conisation to index sampling was 188 days (IQR, 172–213; range, 90–541) in this index cohort.

All 2395 index samples had a valid HPV DNA result, while 2326 (97.1%) had evaluable cytology. An mRNA record was available for 2210 samples (92.3%), of which 2200 had valid results and 10 were invalid; 185 samples had no linked mRNA record. The common

analytical cohort comprised 2136 women (89.2% of the index cohort) with valid results for all three testing modalities (Table 1). This cohort was used consistently for all six testing strategies and the ATHENA-derived referral classification. The median age at conisation was 32 years (IQR, 27–43; range, 19–83).

Table 1. Study population and availability of post-treatment test results.

Cohort or Measure	*n*	%	Denominator
Women treated by conisation, 2016–2020	2625	100.0	2625
Represented in cytology/HPV dataset	2565	97.7	2625
Eligible index sample with valid HPV DNA result	2395	91.2	2625
Index samples with evaluable cytology	2326	97.1	2395
Index samples with linked mRNA record	2210	92.3	2395
Index samples with valid mRNA result	2200	91.9	2395
Common analytical cohort	2136	89.2	2395
Excluded from common analytical cohort	259	10.8	2395

Notes: The baseline population comprised the first conisation per woman during 2016–2020. All 2395 index samples had valid HPV DNA results. Cytology and mRNA availability represent overlapping subsets rather than sequential exclusions. Among the 259 women excluded from the common analytical cohort, 195 lacked a valid mRNA result (185 without a linked record and 10 with an invalid result), 69 had inadequate cytology, and five met both criteria. Percentages were calculated using the denominators indicated.

Of the 259 women excluded from the common analytical cohort, 195 lacked a valid mRNA result and 69 had inadequate cytology; five women met both exclusion criteria. Inclusion was strongly associated with the calendar year of the index visit. Among women with index visits during 2016–2020, the proportion included ranged from 95.2% to 97.3%, whereas none of the 181 women with index visits in 2021 had complete results for all three testing modalities. Consequently, women treated in 2020 accounted for 185 of the 259 excluded women (71.4%), compared with 190 of the 2136 included women (8.9%).

The median age at conisation among excluded women was 33 years (IQR, 28–44; range, 22–76). The proportions with registered CIN2+ were 7.6% (162/2136) among included women and 6.9% (18/259) among excluded women. Corresponding proportions were 3.5% (74/2136) and 3.9% (10/259) for CIN3+, and 0.3% (6/2136) and 0.4% (1/259) for invasive cervical cancer. Characteristics and registered outcomes of included and excluded women are presented in Table 2. These comparisons were descriptive and should be interpreted in the context of differences in calendar-year distribution and available observation periods.

3.2. Available Administrative Follow-Up and Timing of Histological Endpoints

In the common analytical cohort of 2136 women, the median available administrative follow-up from the index visit to 31 December 2025 was 7.24 years (IQR, 6.19–8.28; range, 5.00–9.72). This interval represented the potential observation period rather than the duration of documented clinical surveillance.

Among the 162 women with registered CIN2+, the median interval from the index visit to the recorded CIN2+ specimen date was 208 days (IQR, 73–931). CIN2+ was registered within 365 days in 91 women (56.2%) and more than 730 days after the index visit in 49 women (30.2%). Among the 74 women with registered CIN3+, the median interval to the recorded CIN3+ specimen date was 139 days (IQR, 61–540). CIN3+ was registered within 365 days in 49 women (66.2%) and more than 730 days after the index visit in

15 women (20.3%). These intervals reflect the timing of histological ascertainment and do not establish when the lesions first developed.

Table 2. Characteristics and registered outcomes of women included in and excluded from the common analysis cohort.

Characteristic	Common Analytical Cohort (n = 2136)	Excluded from Common Analytical Cohort (n = 259)
Age at conisation, median (IQR), years	32 (27–43)	33 (28–44)
Age at conisation, range, years	19–83	22–76
Conisation year, *n* (%)		
2016	437 (20.5)	24 (9.3)
2017	506 (23.7)	15 (5.8)
2018	521 (24.4)	20 (7.7)
2019	482 (22.6)	15 (5.8)
2020	190 (8.9)	185 (71.4)
Index visit year, *n* (%)		
2016	198 (9.3)	10 (3.9)
2017	470 (22.0)	22 (8.5)
2018	525 (24.6)	16 (6.2)
2019	511 (23.9)	18 (6.9)
2020	432 (20.2)	12 (4.6)
2021	0 (0.0)	181 (69.9)
Registered outcomes through 2025, *n* (%)		
CIN2+	162 (7.6)	18 (6.9)
CIN3+	74 (3.5)	10 (3.9)
Invasive cervical cancer	6 (0.3)	1 (0.4)

Notes: All index conisations were performed using the loop electrosurgical excision procedure (LEEP). Included women had valid cytology, HPV DNA and HPV16/18/45 E6/E7 mRNA results at the index visit. Percentages were calculated within each group. Histological outcomes were recorded from the index visit through 31 December 2025 and were not mutually exclusive. Women without a registered endpoint were not necessarily histologically verified as disease-free. Comparisons were descriptive, and the groups differed in calendar-year distribution and available observation periods. Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; IQR, interquartile range.

3.3. Test Positivity of the Surveillance Strategies

Among the 2136 women in the common analytical cohort, 583 (27.3%) had ASC-US+ cytology and 119 (5.6%) had high-grade+ cytology at the index post-treatment visit. HPV DNA positivity was observed in 522 women (24.4%), whereas HPV16/18/45 E6/E7 mRNA positivity was observed in 156 (7.3%) (Table 3).

Combining ASC-US+ cytology with mRNA positivity using an inclusive OR rule classified 617 women (28.9%) as test-positive, an increase of 34 women (1.6 percentage points) compared with ASC-US+ cytology alone. Combining high-grade+ cytology with mRNA positivity classified 223 women (10.4%) as test-positive, an increase of 104 women (4.9 percentage points) compared with high-grade+ cytology alone. The high-grade+ cytology OR mRNA strategy classified 299 fewer women as test-positive than HPV DNA testing (10.4% versus 24.4%) and 394 fewer than ASC-US+ cytology OR mRNA testing (10.4% versus 28.9%).

3.4. Performance of the Surveillance Strategies for CIN2+

Histologically confirmed CIN2+ was registered in 162 of the 2136 women (7.6%) during follow-up. HPV DNA testing had the highest observed sensitivity (89.5%), with

a specificity of 80.9%, positive predictive value (PPV) of 27.8% and negative predictive value (NPV) of 98.9% (Table 4). Compared with ASC-US+ cytology, HPV DNA testing had numerically higher sensitivity (89.5% versus 84.0%; McNemar $p = 0.108$) and significantly higher specificity (80.9% versus 77.4%; $p < 0.001$).

Table 3. Test positivity of six surveillance strategies at the index post-treatment visit ($N = 2136$).

Surveillance Strategy	Test-Positive, *n*	Test-Positive, %
ASC-US+ cytology	583	27.3
High-grade+ cytology	119	5.6
HPV DNA positive	522	24.4
HPV16/18/45 E6/E7 mRNA positive	156	7.3
ASC-US+ cytology OR mRNA positive	617	28.9
High-grade+ cytology OR mRNA positive	223	10.4

Notes: All strategies were evaluated in the same 2136 women with valid cytology, HPV DNA and HPV16/18/45 E6/E7 mRNA results. ASC-US+ includes ASC-US and more severe cytological abnormalities. High-grade+ comprises AGUS/AGC, ASC-H, HSIL, AIS and cytological malignancy, as operationally defined in Section 2.4. HPV DNA positivity was defined as detection of at least one of the 14 high-risk HPV genotypes targeted by the cobas 4800 HPV Test. mRNA positivity was defined as detection of E6/E7 mRNA from HPV16, HPV18 or HPV45 using PreTect® SEE. Combined strategies applied an inclusive OR rule. Strategies were evaluated in overlapping groups and their counts should not be summed. Test positivity does not represent observed colposcopy referral rates.

Table 4. Performance of six surveillance strategies at the index post-treatment visit for histologically confirmed CIN2+ registered during follow-up through 2025 ($N = 2136$).

Surveillance Strategy	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
ASC-US+ cytology	84.0 (77.5–88.8)	77.4 (75.5–79.1)	23.3 (20.1–26.9)	98.3 (97.6–98.9)
High-grade+ cytology	43.2 (35.8–50.9)	97.5 (96.7–98.1)	58.8 (49.8–67.3)	95.4 (94.4–96.3)
HPV DNA positive	89.5 (83.8–93.3)	80.9 (79.1–82.6)	27.8 (24.1–31.8)	98.9 (98.3–99.3)
HPV16/18/45 E6/E7 mRNA-positive	45.7 (38.2–53.4)	95.8 (94.9–96.6)	47.4 (39.8–55.2)	95.6 (94.6–96.4)
ASC-US+ cytology OR mRNA positivity	88.9 (83.1–92.9)	76.0 (74.1–77.9)	23.3 (20.2–26.8)	98.8 (98.1–99.2)
High-grade+ cytology OR mRNA positivity	67.9 (60.4–74.6)	94.3 (93.2–95.2)	49.3 (42.8–55.8)	97.3 (96.5–97.9)

Notes: All strategies were evaluated in the common analytical cohort of 2136 women, including 162 with registered CIN2+ and 1974 without a registered CIN2+ endpoint. Histological outcomes were ascertained at or after the index visit through 31 December 2025. Sensitivity, specificity, PPV and NPV were calculated relative to registered histological outcomes, and 95% confidence intervals were calculated using the Wilson method. Women without a registered endpoint were not uniformly verified by histology. Combined cytology/mRNA strategies applied an inclusive OR rule. Cytological positivity thresholds and assay definitions are provided in Sections 2.4–2.7. Abbreviations: CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse, including adenocarcinoma in situ and invasive cervical cancer; HPV, human papillomavirus; mRNA, messenger RNA; NPV, negative predictive value; PPV, positive predictive value.

Combining ASC-US+ cytology with HPV16/18/45 E6/E7 mRNA positivity increased sensitivity from 84.0% to 88.9% compared with ASC-US+ cytology alone ($p = 0.008$), identifying eight additional women with registered CIN2+. This increase was accompanied by a reduction in specificity from 77.4% to 76.0% ($p < 0.001$). The combined strategy had a PPV of 23.3% and an NPV of 98.8%. Its sensitivity did not differ significantly from that of

HPV DNA testing (88.9% versus 89.5%; $p = 1.000$), whereas its specificity was significantly lower (76.0% versus 80.9%; $p < 0.001$).

High-grade+ cytology and HPV16/18/45 E6/E7 mRNA testing alone had sensitivities of 43.2% and 45.7%, respectively, with specificities of 97.5% and 95.8%. Combining high-grade+ cytology with mRNA positivity increased sensitivity to 67.9% ($p < 0.001$), identifying 40 additional women with registered CIN2+ compared with high-grade+ cytology alone. Specificity decreased from 97.5% to 94.3% ($p < 0.001$). The combined strategy had a PPV of 49.3% and an NPV of 97.3%. Compared with HPV DNA testing, this strategy had significantly lower sensitivity (67.9% versus 89.5%) but higher specificity (94.3% versus 80.9%; both $p < 0.001$).

3.5. Performance of the Surveillance Strategies for CIN3+

Histologically confirmed CIN3+ was registered in 74 of the 2136 women (3.5%) during follow-up. HPV DNA testing had a sensitivity of 93.2%, specificity of 78.0%, positive predictive value (PPV) of 13.2% and negative predictive value (NPV) of 99.7% (Table 5). Compared with ASC-US+ cytology, HPV DNA testing showed no statistically significant difference in sensitivity (93.2% versus 91.9%; McNemar $p = 1.000$), whereas specificity was significantly higher (78.0% versus 75.0%; $p < 0.001$).

Table 5. Performance of six surveillance strategies at the index post-treatment visit for histologically confirmed CIN3+ registered during follow-up through 2025 ($N = 2136$).

Surveillance Strategy	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
ASC-US+ cytology	91.9 (83.4–96.2)	75.0 (73.1–76.8)	11.7 (9.3–14.5)	99.6 (99.2–99.8)
High-grade+ cytology	60.8 (49.4–71.1)	96.4 (95.5–97.1)	37.8 (29.6–46.8)	98.6 (97.9–99.0)
HPV DNA positive	93.2 (85.1–97.1)	78.0 (76.2–79.8)	13.2 (10.6–16.4)	99.7 (99.3–99.9)
HPV16/18/45 E6/E7 mRNA positivity	54.1 (42.8–64.9)	94.4 (93.3–95.3)	25.6 (19.4–33.0)	98.3 (97.6–98.8)
ASC-US+ cytology OR mRNA positivity	95.9 (88.7–98.6)	73.5 (71.6–75.4)	11.5 (9.2–14.3)	99.8 (99.4–99.9)
High-grade+ cytology OR mRNA positivity	82.4 (72.2–89.4)	92.1 (90.9–93.2)	27.4 (21.9–33.6)	99.3 (98.8–99.6)

Notes: All strategies were evaluated in the common analytical cohort of 2136 women, including 74 with registered CIN3+ and 2062 without a registered CIN3+ endpoint. Histological outcomes were ascertained at or after the index visit through 31 December 2025. Sensitivity, specificity, PPV and NPV were calculated relative to registered histological outcomes, with Wilson 95% confidence intervals. Women without a registered endpoint were not uniformly verified by histology. Combined cytology/mRNA strategies applied an inclusive OR rule. Cytological positivity thresholds and assay definitions are provided in Sections 2.4–2.7. Abbreviations: CI, confidence interval; CIN3+, cervical intraepithelial neoplasia grade 3 or worse, including adenocarcinoma in situ and invasive cervical cancer; HPV, human papillomavirus; mRNA, messenger RNA; NPV, negative predictive value; PPV, positive predictive value.

Combining ASC-US+ cytology with HPV16/18/45 E6/E7 mRNA positivity yielded the highest observed sensitivity (95.9%), identifying 71 of the 74 women with registered CIN3+, including three additional cases compared with ASC-US+ cytology alone. The increase in sensitivity was not statistically significant compared with either ASC-US+ cytology ($p = 0.250$) or HPV DNA testing ($p = 0.687$). The combined strategy had a specificity of 73.5%, PPV of 11.5% and NPV of 99.8%. Specificity was significantly lower than that of both ASC-US+ cytology and HPV DNA testing ($p < 0.001$ for both comparisons).

High-grade+ cytology and HPV16/18/45 E6/E7 mRNA testing alone had sensitivities of 60.8% and 54.1%, respectively, with specificities of 96.4% and 94.4%. Combin-

ing high-grade+ cytology with mRNA positivity increased sensitivity from 60.8% to 82.4% ($p < 0.001$), identifying 16 additional women with registered CIN3+ compared with high-grade+ cytology alone. Specificity decreased from 96.4% to 92.1% ($p < 0.001$). The combined strategy had a PPV of 27.4% and an NPV of 99.3%.

Compared with HPV DNA testing, the high-grade+ cytology OR mRNA strategy had numerically lower sensitivity (82.4% versus 93.2%; $p = 0.077$) and significantly higher specificity (92.1% versus 78.0%; $p < 0.001$). Compared with ASC-US+ cytology OR mRNA testing, it had significantly lower sensitivity (82.4% versus 95.9%; $p = 0.002$) and higher specificity (92.1% versus 73.5%; $p < 0.001$).

3.6. Risk Stratification by Cytology and HPV16/18/45 E6/E7 mRNA Status

The proportions of women with registered CIN2+ and CIN3+ differed according to cytology and HPV16/18/45 E6/E7 mRNA status at the index post-treatment visit (Table 6). Among women with NILM cytology, CIN2+ was registered in 18/1519 (1.2%) mRNA-negative women and 8/34 (23.5%) mRNA-positive women. Corresponding proportions for CIN3+ were 3/1519 (0.2%) and 3/34 (8.8%). The risk ratios (RRs) comparing mRNA-positive with mRNA-negative women were 19.86 (95% CI, 9.28–42.47) for CIN2+ and 44.68 (95% CI, 9.35–213.42) for CIN3+. These estimates had wide confidence intervals, particularly for CIN3+, reflecting the small number of registered endpoints.

Table 6. Histologically confirmed CIN2+ and CIN3+ registered during follow-up according to cytology and HPV16/18/45 mRNA status at the index post-treatment visit ($N = 2136$).

Cytology	mRNA Status	Women, *n*	CIN2+, *n*/*N* (%) (95% CI)	CIN3+, *n*/*N* (%) (95% CI)
NILM	Negative	1519	18/1519; 1.2 (0.8–1.9)	3/1519; 0.2 (0.1–0.6)
NILM	Positive	34	8/34; 23.5 (12.4–40.0)	3/34; 8.8 (3.0–23.0)
ASC-US/LSIL	Negative	394	34/394; 8.6 (6.2–11.8)	10/394; 2.5 (1.4–4.6)
ASC-US/LSIL	Positive	70	32/70; 45.7 (34.6–57.3)	13/70; 18.6 (11.2–29.2)
High-grade+	Negative	67	36/67; 53.7 (41.9–65.1)	21/67; 31.3 (21.5–43.2)
High-grade+	Positive	52	34/52; 65.4 (51.8–76.8)	24/52; 46.2 (33.3–59.5)

Notes: The six mutually exclusive groups comprise the common analytical cohort of 2136 women. Endpoint proportions represent histologically confirmed CIN2+ and CIN3+ registered at or after the index visit through 31 December 2025, rather than time-to-event estimates over a uniform follow-up period. Wilson 95% confidence intervals were calculated for endpoint proportions. Women without a registered endpoint were not uniformly verified by histology. CIN2+ includes CIN2, CIN3, adenocarcinoma in situ (AIS) and invasive cervical cancer; CIN3+ includes CIN3, AIS and invasive cervical cancer. High-grade+ cytology was defined as AGUS/AGC, ASC-H, HSIL, AIS or cytological malignancy. mRNA positivity denotes HPV16/18/45 E6/E7 mRNA detection using PreTect® SEE. Abbreviations: CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; mRNA, messenger RNA; NILM, negative for intraepithelial lesion or malignancy.

Among women with ASC-US/LSIL cytology, CIN2+ was registered in 34/394 (8.6%) mRNA-negative women compared with 32/70 (45.7%) mRNA-positive women (RR, 5.30; 95% CI, 3.51–7.99). Corresponding proportions for CIN3+ were 10/394 (2.5%) and 13/70 (18.6%), respectively (RR, 7.32; 95% CI, 3.34–16.03).

Among women with high-grade+ cytology, the proportions with registered CIN2+ and CIN3+ were elevated irrespective of mRNA status. CIN2+ was registered in 36/67 (53.7%) mRNA-negative women and 34/52 (65.4%) mRNA-positive women, while CIN3+ was registered in 21/67 (31.3%) and 24/52 (46.2%), respectively. The corresponding RRs were 1.22 (95% CI, 0.90–1.64) for CIN2+ and 1.47 (95% CI, 0.93–2.33) for CIN3+. Neither confidence interval excluded unity.

3.7. Performance of the ATHENA-Derived Referral Strategy

The ATHENA-derived referral classification identified 415 of 2136 women (19.4%) as referral-positive, comprising 173 women with HPV16/18 DNA positivity and 242 with positivity for other high-risk HPV DNA types combined with ASC-US+ cytology (Table 7). The remaining 1721 women were classified as referral-negative, including 1614 who were HPV DNA-negative and 107 with positivity for other high-risk HPV DNA types and NILM cytology. Compared with HPV DNA positivity alone, the ATHENA-derived strategy classified 107 fewer women as positive.

Table 7. Histologically confirmed CIN2+ and CIN3+ registered during follow-up according to the ATHENA-derived risk/referral strategy at the index post-treatment visit ($N = 2136$).

ATHENA-Derived Group	Referral Classification	Women, *n*	CIN2+, *n*/*N*; % (95% CI)	CIN3+, *n*/*N*; % (95% CI)
HPV DNA-negative	Negative	1614	17/1614; 1.1 (0.7–1.7)	5/1614; 0.3 (0.1–0.7)
HPV16/18 DNA-positive	Positive	173	83/173; 48.0 (40.7–55.4)	41/173; 23.7 (18.0–30.6)
Other HPV DNA-positive/ASC-US+	Positive	242	54/242; 22.3 (17.5–28.0)	26/242; 10.7 (7.4–15.3)
Other HPV DNA-positive/NILM	Negative	107	8/107; 7.5 (3.8–14.1)	2/107; 1.9 (0.5–6.6)

Notes: The four mutually exclusive groups comprise the common analytical cohort of 2136 women. Referral-positive classification was defined as HPV16/18 DNA positivity irrespective of cytology, or positivity for other high-risk HPV DNA types combined with ASC-US+ cytology. Women with negative HPV DNA results or other high-risk HPV DNA positivity and NILM cytology were classified as referral-negative. For CIN2+, sensitivity, specificity, PPV and NPV were 84.6% (78.2–89.3), 85.9% (84.3–87.4), 33.0% (28.7–37.7) and 98.5% (97.9–99.0), respectively. Corresponding estimates for CIN3+ were 90.5% (81.7–95.3), 83.1% (81.4–84.7), 16.1% (12.9–20.0) and 99.6% (99.2–99.8). All confidence intervals were calculated using the Wilson method. Histological endpoints were registered at or after the index visit through 31 December 2025. Endpoint proportions do not represent cumulative incidence at a uniform follow-up duration, and women without registered endpoints were not uniformly verified by histology. The ATHENA-derived strategy was evaluated retrospectively as an exploratory classification and was not validated as a post-treatment management algorithm. Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; NPV, negative predictive value; PPV, positive predictive value.

For CIN3+, the ATHENA-derived strategy had a sensitivity of 90.5%, specificity of 83.1%, positive predictive value (PPV) of 16.1% and negative predictive value (NPV) of 99.6%. Sensitivity was numerically lower than that of HPV DNA testing (90.5% versus 93.2%; McNemar $p = 0.500$), whereas specificity was significantly higher (83.1% versus 78.0%; $p < 0.001$). Compared with ASC-US+ cytology OR mRNA positivity, sensitivity was also numerically lower (90.5% versus 95.9%; $p = 0.125$), while specificity was significantly higher (83.1% versus 73.5%; $p < 0.001$). Compared with high-grade+ cytology OR mRNA positivity, sensitivity was numerically higher (90.5% versus 82.4%; $p = 0.180$), whereas specificity was significantly lower (83.1% versus 92.1%; $p < 0.001$).

For CIN2+, the ATHENA-derived strategy had a sensitivity of 84.6%, specificity of 85.9%, PPV of 33.0% and NPV of 98.5%. Sensitivity was significantly lower than that of HPV DNA testing (89.5%; $p = 0.008$) and ASC-US+ cytology OR mRNA positivity (88.9%; $p = 0.039$), but higher than that of high-grade+ cytology OR mRNA positivity (67.9%; $p < 0.001$). Specificity was significantly higher than that of HPV DNA testing and ASC-US+ cytology OR mRNA positivity, but lower than that of high-grade+ cytology OR mRNA positivity (all $p < 0.001$).

Registered endpoint proportions varied across the four ATHENA-derived groups. Among HPV16/18 DNA-positive women, CIN2+ was registered in 83/173 (48.0%) and CIN3+ in 41/173 (23.7%). Among women positive for other high-risk HPV DNA types with ASC-US+ cytology, corresponding proportions were 54/242 (22.3%) and 26/242 (10.7%). In the other HPV DNA-positive/NILM group, classified as referral-negative, CIN2+ was registered in 8/107 women (7.5%) and CIN3+ in 2/107 (1.9%). Among HPV DNA-negative women, corresponding proportions were 17/1614 (1.1%) and 5/1614 (0.3%).

All six women with invasive cervical cancer registered during follow-up were classified as referral-positive at the index visit: four were HPV16/18 DNA-positive and two were positive for other high-risk HPV DNA types with ASC-US+ cytology.

3.8. Invasive Cervical Cancer Cases

Six invasive cervical cancers were registered during follow-up, comprising five squamous cell carcinomas and one adenocarcinoma (Table 8). All six women were HPV DNA-positive and had ASC-US+ cytology at the index post-treatment visit. Four were HPV16 DNA-positive with corresponding HPV16 E6/E7 mRNA positivity, whereas the remaining two were positive for other high-risk HPV DNA types and negative for HPV16/18/45 E6/E7 mRNA. None was HPV18 DNA-positive.

Table 8. Characteristics of the six invasive cervical cancers registered during follow-up after the index post-treatment visit ($N = 6$).

Case	Age at Conisation (Years)	Histology	Index Cytology	HPV DNA Result	HPV16/18/45 mRNA Result	Time to Cancer (Days)
1	57	Adenocarcinoma	ASC-US	HPV16-positive	HPV16-positive	112
2	66	SCC	HSIL	HPV16-positive	HPV16-positive	27
3	66	SCC	HSIL	Other HR-HPV-positive	Negative	66
4	59	SCC	HSIL	HPV16-positive	HPV16-positive	1084
5	47	SCC	HSIL	HPV16-positive	HPV16-positive	273
6	43	SCC	ASC-H	Other HR-HPV-positive	Negative	1147

Notes: Cases are numbered for presentation only. All six women were HPV DNA-positive and were classified as referral-positive by the ATHENA-derived strategy at the index visit. The cobas 4800 HPV Test reports HPV16 and HPV18 DNA individually and provides a single pooled result for 12 other genotypes. In cases 3 and 6, "Other HR-HPV-positive" indicates that HPV16 and HPV18 DNA were negative and that one or more genotypes in the pool were detected. The individual genotypes in the pool remain undetermined. HPV16/18/45 mRNA results indicate genotype-specific E6/E7 mRNA detection using PreTect® SEE. Time to cancer was defined as the interval from the collection date of the first eligible post-conisation follow-up sample (the index sample) to the collection date of the specimen in which invasive cervical cancer was histologically confirmed. Abbreviations: ASC-H, atypical squamous cells, cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; mRNA, messenger RNA; SCC, squamous cell carcinoma.

All five women subsequently diagnosed with squamous cell carcinoma had high-grade+ cytology at the index visit, comprising four HSIL results and one ASC-H result. The woman subsequently diagnosed with adenocarcinoma had ASC-US cytology and was HPV16 DNA- and mRNA-positive. Both combined cytology/mRNA strategies classified all six women as test-positive, as did the ATHENA-derived referral classification. High-grade+

cytology alone classified five women as positive, whereas HPV16/18/45 E6/E7 mRNA testing alone classified four.

The median interval from the index visit to histological cancer diagnosis was 192.5 days (range, 27–1147). Four cancers were diagnosed within one year of the index visit and two more than two years after the index visit. No invasive cancers were registered among HPV DNA-negative women or among women positive for other high-risk HPV DNA types with NILM cytology. Findings were interpreted descriptively because of the small number of cancer cases.

4. Discussion

4.1. Principal Findings

This retrospective regional cohort study demonstrates that results obtained at the first post-treatment visit can stratify the risk of histologically confirmed cervical disease registered during subsequent follow-up. The evaluated strategies differed in their balance between sensitivity, specificity and test positivity. HPV DNA testing provided high sensitivity for CIN3+, whereas combining ASC-US+ cytology with HPV16/18/45 E6/E7 mRNA positivity achieved the highest observed sensitivity. Importantly, adding mRNA testing to high-grade+ cytology substantially improved sensitivity while maintaining high specificity and a considerably lower proportion of positive classifications than HPV DNA testing. The exploratory ATHENA-derived referral classification provided an additional comparison of alternative risk-stratification approaches.

A principal finding was the additional risk discrimination provided by HPV16/18/45 E6/E7 mRNA detection within cytological categories. Among women with NILM or ASC-US/LSIL cytology, mRNA positivity identified subgroups with markedly higher proportions of registered CIN2+ and CIN3+, despite negative or low-grade cytological findings. Conversely, women with high-grade+ cytology had substantial proportions of registered disease irrespective of mRNA status. Together, these findings highlight the potential value of genotype-specific E6/E7 mRNA testing as a complementary marker to cervical cytology in post-treatment risk stratification.

4.2. Interpretation in Relation to Previous Studies

Previous studies have highlighted the importance of both post-treatment risk assessment and appropriate clinical follow-up [17,18]. In a Dutch multi-cohort study, Kocken et al. evaluated long-term outcomes after treatment for CIN2/3 and demonstrated that negative HPV DNA and cytology co-tests at 6 and 24 months identified women with a substantially lower subsequent risk of high-grade disease [17]. Nevertheless, some CIN2+ diagnoses occurred during extended follow-up, illustrating that reassuring post-treatment results do not eliminate longer-term risk. In Northern Norway, Skjeldestad and Sørbye similarly reported a low risk of CIN3+ among women with negative HPV results and normal or low-grade cytology at the first follow-up visit, while identifying substantial gaps in adherence to recommended surveillance [18]. Together, these studies emphasise the importance of interpreting post-treatment test results within a longitudinal surveillance pathway that combines risk assessment with reliable attendance and appropriate investigation of abnormal findings.

During the study period, cervical screening in Troms and Finnmark was initially cytology-based, with reflex HPV DNA testing for ASC-US or LSIL. From January 2019, primary HPV DNA screening was introduced gradually among women aged 34–69 years, with reflex cytology for HPV-positive samples. After conisation, the Norwegian Cervical Cancer Screening Programme recommended HPV DNA and cytology co-testing at 6 and 12 months, with subsequent management guided by the co-test results [18]. At UNN, three-

genotype HPV mRNA testing was also performed on residual cervical cytology material as part of a local quality assurance project aimed at reducing the risk of cervical cancer after normal cytology [8].

The potential value of HPV E6/E7 mRNA testing after excisional treatment has been investigated in several studies using genotype-specific assays [11,12,19–21]. Tropé et al. reported limited sensitivity for residual CIN2+ using the five-genotype PreTect® HPV-Proofer assay, with sensitivity of 45.5% at six months after conisation [19]. Frega et al., using the same assay, reported higher mRNA sensitivity for recurrent than residual disease (73.5% versus 52%), illustrating the importance of endpoint definitions and follow-up timing [20]. Zappacosta et al. reported higher specificity and positive predictive value for mRNA testing than for combined cytology and HPV DNA testing [21], while Carcea et al. reported associations between postoperative E6/E7 mRNA positivity and treatment-related outcomes [11,12]. These heterogeneous findings support further investigation of genotype-specific E6/E7 mRNA detection in post-treatment surveillance, while indicating that performance varies across study settings and endpoint definitions. The broader five-genotype coverage of HPV-Proofer compared with the three-genotype PreTect® SEE assay used in the present study should also be considered when interpreting differences in test performance.

Systematic reviews and meta-analyses have further evaluated HPV E6/E7 mRNA testing after conisation [22,23]. Macedo et al., in a meta-analysis of five studies including 1148 women, reported a pooled sensitivity of 62.4% and specificity of 91.9% for CIN2+ detection. In the subset of four studies comprising 1090 women with directly comparable test results, HPV mRNA testing showed higher pooled specificity than HPV DNA testing (91.5% versus 72.4%), but lower pooled sensitivity (61.3% versus 86.8%) [22]. More recently, Zhao et al. reported higher specificity for HPV E6/E7 mRNA testing than for HPV DNA testing in post-conisation surveillance (relative specificity 1.28; 95% CI 1.22–1.34), including a specificity advantage for PreTect HPV-Proofer [23]. However, the pooled relative sensitivity was 0.84 (95% CI 0.70–1.00), indicating that improved specificity may be accompanied by reduced sensitivity. These findings support further evaluation of mRNA-based risk stratification after treatment, with careful consideration of assay-specific genotype coverage and the balance between sensitivity and specificity.

The 14-genotype Aptima HPV mRNA assay has also been evaluated after excisional treatment. In a Scottish retrospective case–control study, Bhatia et al. [24] reported sensitivities for CIN2+ of 96.8% with Aptima and 93.7% with the Abbott RealTime HPV DNA assay. In an Italian study, Bottari et al. [25] found that Aptima was positive in 27 of 86 paired samples (31.4%) at 3–6 months after treatment, compared with 33 of 86 (38.4%) for Hybrid Capture 2. Differences in genotype coverage, study design and outcome assessment limit direct comparison with our evaluation of cytology and three-genotype HPV mRNA co-testing for long-term CIN3+ risk.

The present study extends this evidence by evaluating the three-genotype PreTect® SEE assay (HPV16/18/45) alongside cervical cytology and HPV DNA within the same regional post-conisation cohort. A particular contribution is the assessment of additional risk discrimination by mRNA status within cytological categories and the direct comparison of alternative surveillance strategies in women with valid results for all three testing modalities. These analyses complement previous investigations of three-genotype cytology/mRNA co-testing in routine clinical practice and seven-genotype mRNA triage among HPV DNA-positive screening participants [8,26]. During 2016–2020, all cervical cytology samples processed at UNN underwent PreTect® SEE testing irrespective of the indication for sampling or concurrent HPV DNA testing. Consequently, the present cohort constitutes a post-treatment subpopulation of the broader routine-practice cohort reported previously,

with shared index samples [8]. The present analysis addresses the distinct clinical question of surveillance after treatment for CIN2/3, with one index visit per woman and paired comparison of HPV DNA, cytology and mRNA-based strategies. The treatment period also overlapped with that of the earlier Northern Norwegian post-treatment cohort (2014–2017), creating the possibility of shared participants [18]. These regional studies therefore provide complementary evidence and should not be regarded as independent validation.

4.3. Complementary Information from Cytology and HPV mRNA

Cervical cytology and HPV E6/E7 mRNA testing provide complementary information on HPV-associated cervical disease. Cytology identifies morphological abnormalities reflecting cellular changes [14], whereas genotype-specific mRNA testing detects transcription of the viral E6/E7 oncogenes, which play a central role in HPV-mediated carcinogenesis [7]. Detection of these transcripts may therefore provide additional molecular information about viral oncogene expression that is not fully captured by cytomorphological assessment. Conversely, cytology can identify abnormalities associated with HPV genotypes not targeted by the mRNA assay. High-grade squamous or glandular cytological abnormalities may warrant clinical investigation despite a negative HPV result [4]; a negative result from a restricted-genotype mRNA assay should therefore not override such findings.

The present findings illustrate the value of interpreting these two modalities together. Among women with NILM cytology, HPV16/18/45 mRNA positivity was associated with substantially higher proportions of registered CIN2+ and CIN3+ than mRNA negativity, indicating that morphologically negative findings did not necessarily correspond to low disease risk when oncogenic transcripts were detected. Similarly, among women with ASC-US/LSIL cytology, mRNA positivity identified a subgroup with markedly elevated proportions of high-grade disease. These findings suggest that genotype-specific E6/E7 mRNA detection may improve risk discrimination among women with negative or low-grade cytological findings. In contrast, women with high-grade+ cytology had substantial proportions of registered CIN2+ and CIN3+ irrespective of mRNA status, demonstrating the continued importance of cytological abnormalities in identifying women requiring further clinical assessment.

The interpretation of mRNA performance must also consider assay genotype coverage. PreTect® SEE detects E6/E7 transcripts from HPV16, HPV18 and HPV45 [8], whereas the HPV DNA assay used in this study covers 14 genotypes. Consequently, differences in sensitivity cannot be attributed solely to detection of viral DNA versus oncogene transcription. A negative PreTect® SEE result does not exclude E6/E7 transcription from other oncogenic HPV genotypes, nor does a positive result independently establish the presence of a transforming lesion. The seven-genotype PreTect HPV-Proofer'7 assay extends coverage to HPV16, HPV18, HPV31, HPV33, HPV45, HPV52 and HPV58 [26]. Genotype-specific risk stratification is increasingly incorporated into cervical screening guidelines, although current recommendations for extended HPV DNA genotyping do not establish the clinical utility of genotype-specific mRNA testing after treatment [27]. In a primary-screening cohort of HPV DNA-positive women, seven-genotype mRNA testing showed higher specificity than cytology-based triage [26]. Whether this broader mRNA genotype coverage could improve risk stratification following conisation requires evaluation in post-treatment populations.

The invasive cancer cases provide further descriptive support for the complementary information obtained from cytology and mRNA testing. The woman subsequently diagnosed with adenocarcinoma had ASC-US cytology and positive HPV16 DNA and mRNA results, whereas two women subsequently diagnosed with squamous cell carcinoma had high-grade+ cytology, positivity for other oncogenic HPV DNA types and negative HPV16/18/45 mRNA results. Both combined cytology/mRNA strategies classified all six

women with subsequently registered invasive cancer as test-positive. Although the small number of cases precludes reliable conclusions regarding cancer detection performance, these observations illustrate how molecular and morphological findings may contribute complementary information in post-treatment surveillance.

4.4. A Potential Risk-Stratified Post-Treatment Pathway

The complementary information provided by cervical cytology and HPV16/18/45 E6/E7 mRNA testing offers a basis for prospective evaluation of a three-category approach to post-treatment surveillance. Under this proposed framework, women with high-grade+ cytology or positive mRNA would be prioritised for colposcopic assessment, whereas women with ASC-US/LSIL cytology and negative mRNA could be considered for structured repeat testing. Women with NILM cytology and negative mRNA would represent a third category potentially suitable for less intensive surveillance. These proposed pathways require validation of testing intervals and eligibility criteria, including consideration of concurrent HPV DNA results and previous clinical history. Current ASCCP guidelines recommend colposcopy after a positive post-treatment HPV test, and the present findings do not establish the safety of deferring this assessment on the basis of negative three-genotype mRNA results [4,27].

When applied retrospectively to the present cohort, this classification separated women into three groups with markedly different proportions of registered CIN3+. The category proposed for immediate investigation comprised 223 women, of whom 61 (27.4%) had registered CIN3+. Among the 394 women with ASC-US/LSIL cytology and negative mRNA, CIN3+ was registered in 10 (2.5%), compared with three of 1519 women (0.2%) with NILM cytology and negative mRNA. These findings illustrate the potential clinical relevance of distinguishing between women with negative and low-grade cytology rather than considering all women negative under the high-grade+ cytology OR mRNA rule as a single low-risk population. The overall NPV of 99.3% for this combined strategy encompasses these different risk categories.

These proportions reflect histological diagnoses registered during unequal observation periods under routine clinical management. They should not be interpreted as immediate or fixed-duration cumulative risks, or directly compared with guideline thresholds to determine surveillance intervals.

The intermediate-risk group is particularly relevant when considering a surveillance pathway incorporating repeat testing rather than immediate investigation. Although mRNA-negative women with ASC-US/LSIL cytology had substantially lower registered disease proportions than mRNA-positive women within the same cytological category, 34 CIN2+ and 10 CIN3+ endpoints were recorded during follow-up. Effective management of this group would therefore require defined repeat-testing intervals, reliable attendance and appropriate escalation of persistent or worsening abnormalities. Similarly, the low proportion of registered CIN3+ among women with NILM cytology and negative mRNA supports further evaluation of less intensive surveillance, but does not independently establish the safety of extending follow-up intervals. The observed endpoints were ascertained under routine clinical management, potentially influenced by HPV DNA results and subsequent examinations, rather than under the proposed pathway.

The potential implementation of such an approach must also be considered in relation to established post-treatment recommendations. The ASCCP guidelines, updated through 2023, recommend three negative HPV tests or cytology/HPV co-tests at 6, 18 and 30 months after treatment for CIN2+ before extending surveillance to three-year intervals, with long-term surveillance recommended for at least 25 years [4,28]. Although the underlying risk estimates were derived from HPV DNA testing, the guidelines permit

appropriately approved HPV DNA and mRNA assays used according to their regulatory indications [4]. These recommendations cannot, however, be directly transferred to a restricted three-genotype mRNA/cytology strategy. The present findings therefore provide a rationale for prospective evaluation of a risk-stratified pathway, including appropriate testing intervals, adherence to follow-up and detection of clinically significant disease among women assigned to deferred investigation.

4.5. Balancing Disease Detection, Investigation Burden and Resource Utilisation

An important objective of post-treatment surveillance is to maintain effective detection of clinically significant cervical disease while limiting unnecessary colposcopic investigations [4]. In the present cohort, the high-grade+ cytology OR HPV16/18/45 E6/E7 mRNA strategy classified 299 fewer women as test-positive than HPV DNA testing alone, representing a 57.3% relative reduction in positive classifications (10.4% versus 24.4%). Specificity for CIN3+ increased from 78.0% to 92.1%, and positive predictive value increased from 13.2% to 27.4%. These findings suggest that incorporating genotype-specific mRNA detection alongside a higher cytological threshold could reduce the number of women selected for immediate investigation while increasing the proportion with registered high-grade disease among those selected. However, the reduction in positive classifications should not be interpreted as an observed reduction in colposcopy use. The actual effect would depend on the management pathway, including repeat testing and subsequent investigations among women initially classified as negative.

The higher specificity and lower test positivity were accompanied by lower sensitivity for CIN3+. The high-grade+ cytology OR mRNA strategy identified 61 of 74 women with registered CIN3+, compared with 69 identified by HPV DNA testing and 71 by ASC-US+ cytology OR mRNA testing. Although the sensitivity difference between the higher-threshold strategy and HPV DNA testing was not statistically significant, the observed difference of 10.8 percentage points may be clinically important. The potential advantages of fewer positive classifications must therefore be evaluated alongside the consequences of deferring investigation in women with initially negative results. The absence of statistical significance does not establish equivalence in sensitivity or comparable protection against high-grade disease.

The exploratory ATHENA-derived referral classification provided an intermediate balance, classifying 19.4% of women as referral-positive while achieving CIN3+ sensitivity of 90.5% and specificity of 83.1%. This classification was adapted from a primary-screening strategy [13] and should not be interpreted as a validated post-treatment algorithm. Together, these comparisons illustrate how alternative combinations of molecular testing and cytology influence the proportion of women selected for further assessment and the proportion with histologically confirmed disease among those classified as positive. The most appropriate balance will depend not only on diagnostic performance but also on the effectiveness of subsequent surveillance and the clinical consequences of delayed diagnosis.

More selective investigation strategies may offer opportunities to reduce unnecessary procedures, associated discomfort and anxiety, and demand on colposcopy services, consistent with the principle of balancing cancer prevention against harms from overtesting and overtreatment [4]. However, these potential benefits must be considered alongside the costs and consequences of repeat testing, additional surveillance visits and possible delays in diagnosis. The present study did not measure actual referral rates, resource utilisation, patient-reported outcomes or costs, and therefore cannot establish the cost-effectiveness of any evaluated strategy. Formal health-economic evaluation should incorporate initial testing, repeat surveillance, attendance, colposcopy and biopsy, subsequent treatment, and the consequences of delayed CIN3+ or cancer detection. Such analyses would help determine

whether the higher specificity and positive predictive value of the high-grade+ cytology OR mRNA strategy translate into a favourable overall balance of clinical benefit, investigation burden and costs.

4.6. Timing of Disease Registration and Implications for Surveillance

The extended period of available administrative follow-up represents an important feature of the present study, allowing associations between index post-treatment test results and registered histological outcomes to be examined beyond the initial surveillance period. The median available administrative follow-up was 7.24 years. Although most CIN3+ endpoints were registered within one year of the index visit, 20.3% were registered more than two years later. For CIN2+, the corresponding proportion was 30.2%. These findings highlight the relevance of evaluating the prognostic information provided by initial surveillance results over an extended observation period, rather than restricting assessment to lesions diagnosed at the first post-treatment examination.

The timing of histological diagnosis does not necessarily reflect the onset of disease. Later diagnoses may represent persistent or residual lesions detected after a delay, as well as subsequently developing disease. Skjeldestad and Sørbye reported seven cases classified as residual cervical cancer, including two diagnosed during follow-up after repeat conisation had failed to identify invasive disease [18]. These observations illustrate that the date of histological confirmation may not correspond to the onset of disease. Accordingly, the present study uses registered CIN2+ and CIN3+ during follow-up as its endpoints without attempting to distinguish residual from recurrent disease solely on the basis of elapsed time.

This longitudinal perspective is also relevant when interpreting the performance of the evaluated surveillance strategies. Women with negative index test classifications who subsequently had a registered endpoint were classified as false-negative relative to that endpoint, although the lesion may not have been present or detectable at the index visit. Conversely, a positive index result may indicate elevated subsequent disease risk without establishing that histological confirmation would have been possible immediately. The reported sensitivity and specificity therefore describe the relationship between index classifications and disease registered during follow-up, rather than diagnostic accuracy for lesions necessarily present at baseline. These findings support evaluation of post-treatment surveillance as a longitudinal process in which initial risk classification is complemented by appropriately scheduled repeat testing and diagnostic follow-up [4,28].

4.7. Strengths and Limitations

The principal strengths of this study include its regional coverage of Troms and Finnmark in Northern Norway, the linkage of treatment, cervical cytology, HPV testing and histology records at UNN, and the direct comparison of alternative surveillance strategies in the same cohort of 2136 women. Applying all strategies to the same women minimised differences in case mix between comparisons, while paired analyses accounted for the dependence between test classifications. Evaluation of both CIN2+ and CIN3+ provided complementary measures of clinically relevant disease, and the extended period of histological outcome ascertainment enabled assessment of associations beyond the initial post-treatment surveillance period. In addition, the analysis of mRNA status within cytological categories provided a more detailed assessment of risk discrimination than comparisons based solely on overall test positivity or diagnostic performance.

Several limitations should be considered. First, histological verification was performed according to routine clinical indications rather than systematically in all women. Those without a registered endpoint included women with histological findings below the relevant threshold and women without histological sampling. Consequently, differential verification

and incomplete ascertainment may have influenced the estimated sensitivity, specificity and predictive values. Although the extended availability of pathology records facilitated identification of later diagnoses, it did not establish complete clinical follow-up or exclude the possibility of unrecorded disease.

Second, the surveillance strategies were reconstructed retrospectively rather than implemented prospectively. Histological outcomes were ascertained during routine care, in which HPV DNA and cytology results guided follow-up. Subsequent examinations and treatment may also have influenced which outcomes were recorded.

The analysis therefore cannot establish how many colposcopies would have been performed, or which outcomes would have occurred, had each alternative strategy independently determined clinical management.

Third, available administrative follow-up varied between women and was calculated to a common study end date. This measure did not establish continuous surveillance or account for death, migration or interruptions in follow-up. Accordingly, registered endpoint proportions represent observed outcomes during unequal observation periods rather than censoring-adjusted cumulative risks at a uniform time horizon. These estimates should not be interpreted directly against fixed-duration clinical risk thresholds.

Fourth, inclusion in the common analytical cohort required valid results for all three testing modalities and varied substantially by calendar year. No women with index visits in 2021 had complete results, and women treated in 2020 were disproportionately represented among those excluded. Although age distributions and registered endpoint proportions were descriptively similar between included and excluded women, differences in calendar-year distribution and available observation periods mean that selection bias cannot be excluded. A comparable cohort of women treated after 2020 was unavailable because routine PreTect SEE testing had been discontinued. Women treated more recently would also have had a shorter observation period for histological outcomes before the study end date of 31 December 2025.

Fifth, the analysis did not account for potential differences in risk associated with resection margin status, treatment histology, HPV vaccination, immune status or detailed HPV infection history. The relatively broad interval permitted between conisation and index testing may also have contributed to variation in test results and subsequent disease ascertainment. Furthermore, the three-genotype coverage of PreTect® SEE should be considered when interpreting its sensitivity relative to HPV DNA testing, which detects a broader range of oncogenic HPV genotypes.

Finally, the number of registered CIN3+ endpoints was relatively modest, several cytology/mRNA subgroups were small, and only six invasive cancers were identified. Multiple paired comparisons were performed without adjustment for multiplicity, and non-significant differences cannot be interpreted as evidence of equivalence. As the study was conducted within a single regional pathology service, differences in cytological assessment, HPV genotype distribution and surveillance practices may affect generalisability. External validation, ideally incorporating prospective evaluation of defined surveillance pathways, is therefore warranted.

4.8. Implications for Further Evaluation

The observed differences in registered disease proportions across cytology/mRNA categories provide a rationale for prospective evaluation of risk-stratified post-treatment surveillance pathways. Such an evaluation could assess immediate colposcopic investigation for women with high-grade+ cytology or HPV16/18/45 E6/E7 mRNA positivity, structured repeat testing for women with ASC-US/LSIL cytology and negative mRNA, and predefined surveillance intervals for women with NILM cytology and negative mRNA. Eligibility for

deferred investigation or longer testing intervals would require consideration of concurrent HPV DNA results, previous clinical history and safeguards for persistent abnormalities. These pathways should be evaluated against established post-treatment management, as current recommendations do not support deferring indicated colposcopy solely on the basis of negative three-genotype mRNA results [4,27,28]. Evaluation should encompass the complete surveillance pathway, incorporating adherence to follow-up, cumulative CIN3+ detection, time to diagnosis, invasive cancer outcomes, investigation and treatment burden, patient-reported outcomes and healthcare costs.

Host-cell DNA methylation testing may help stratify risk among women who remain HPV-positive after excisional treatment. In a post hoc analysis of 364 women treated for CIN2/3, Dick et al. evaluated cervical samples collected at 6 and 12 months after treatment. ASCL1/LHX8 and FAM19A4/miR124-2 methylation were positive in 13 of 14 and 14 of 14 women with recurrent CIN3, respectively. Both panels had lower positivity among women with recurrent CIN2 [29]. Given the small number of CIN3 cases, prospective studies are needed to determine whether adding methylation testing to HPV DNA testing and cytology can improve post-treatment risk stratification.

The present findings support the potential role of HPV16/18/45 E6/E7 mRNA detection as a complementary marker to cytology for post-treatment risk stratification. Combining mRNA positivity with high-grade+ cytology improved sensitivity compared with high-grade+ cytology alone, while maintaining high specificity and substantially lower test positivity than HPV DNA testing. This approach may offer opportunities to reduce the proportion of women selected for immediate investigation while distinguishing groups requiring different intensities of subsequent surveillance. However, its lower observed sensitivity than HPV DNA testing remains an important consideration. Prospective validation is needed to determine whether this strategy can reduce colposcopy referrals while maintaining timely detection of CIN3+, including invasive cervical cancer.

5. Conclusions

In this regional post-conisation cohort, HPV16/18/45 E6/E7 mRNA testing provided additional risk discrimination within cytological categories, particularly among women with NILM or ASC-US/LSIL cytology. Combining mRNA positivity with high-grade+ cytology substantially improved sensitivity compared with high-grade+ cytology alone, while achieving higher specificity and considerably lower test positivity than HPV DNA testing. Although this greater selectivity was accompanied by lower sensitivity than HPV DNA, the findings highlight the potential value of genotype-specific E6/E7 mRNA detection as a complementary marker for post-treatment risk stratification.

The observed differences in registered disease proportions support further evaluation of risk-stratified surveillance pathways incorporating immediate investigation for women with high-grade+ cytology or mRNA positivity, structured repeat testing for mRNA-negative women with low-grade cytology, and potentially less intensive surveillance for women with NILM cytology and negative mRNA. Prospective evaluation is needed to determine whether such pathways can reduce unnecessary colposcopic investigations while maintaining effective detection of clinically significant cervical disease.

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Institutional Review Board Statement: The Regional Committee for Medical and Health Research Ethics, North Norway (REK Nord), assessed the original project on follow-up after conisation as a quality-assurance project outside the scope of the Norwegian Health Research Act and therefore not subject to REK approval (reference 2015/2497/REK nord; 22 December 2015). The present retrospective analysis was conducted as a continuation of this quality-assurance project, using routinely recorded cervical cytology, HPV testing and histological results. No additional specimen collection, laboratory testing or histological reassessment was performed for this analysis.

Informed Consent Statement: Individual informed consent was not obtained for this retrospective quality-assurance analysis of routinely collected clinical records. The study involved no patient contact or study-specific intervention. Person-specific identifiers were used solely for record linkage and de-duplication within a secure institutional data environment. Only aggregated, non-identifiable results are reported.

Data Availability Statement: The individual-level data supporting this study are not publicly available because they were derived from routine pathology records and are subject to privacy, ethical and institutional data-governance restrictions. Aggregated, non-identifiable data supporting the main analyses, including cross-tabulations of surveillance test results and histological outcomes, may be made available by the corresponding author upon reasonable request and subject to approval by the University Hospital of North Norway.

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Conflicts of Interest: B.M.F. is employed by PreTect AS, the manufacturer of the PreTect® SEE HPV mRNA assay evaluated in this study. PreTect AS provided HPV mRNA testing free of charge during 2016–2019; from 2020, testing was paid for by the Department of Clinical Pathology, University Hospital of North Norway. B.M.F. participated as a scientific collaborator and co-author, contributing to the study and manuscript as described in the Author Contributions statement. Data linkage, endpoint definition and statistical analyses were performed independently at the University Hospital of North Norway. Beyond the provision of HPV mRNA testing and B.M.F.'s declared scientific contributions, PreTect AS had no additional institutional role in the conduct of the study or the decision to publish. S.W.S., M.A. and E.R. declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AGC	Atypical glandular cells
AGUS	Atypical glandular cells of undetermined significance
AI	Artificial intelligence
AIS	Adenocarcinoma in situ
ASC-H	Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion
ASC-US	Atypical squamous cells of undetermined significance
ASCCP	American Society for Colposcopy and Cervical Pathology
ATHENA	Addressing the Need for Advanced HPV Diagnostics
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN2+	Cervical intraepithelial neoplasia grade 2 or worse
CIN3+	Cervical intraepithelial neoplasia grade 3 or worse
DNA	Deoxyribonucleic acid

E6/E7	Human papillomavirus early genes 6 and 7
GPT	Generative pre-trained transformer
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
IQR	Interquartile range
LEEP	Loop electrosurgical excision procedure
LSIL	Low-grade squamous intraepithelial lesion
mRNA	Messenger RNA
NILM	Negative for intraepithelial lesion or malignancy
NPV	Negative predictive value
PPV	Positive predictive value
REK	Regional Committee for Medical and Health Research Ethics (Norway)
RR	Risk ratio
SCC	Squamous cell carcinoma
SPSS	Statistical Package for the Social Sciences
UNN	University Hospital of North Norway

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