



Article

Cervical Cancer Screening: Impact of Human Papillomavirus mRNA Testing on Detecting High-Grade Lesions in Women with Normal Cytology

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Abstract: The human papillomavirus (HPV) is implicated in nearly all cases of cervical cancer. While most HPV infections resolve spontaneously, persistent infections can lead to high-grade lesions and cancer. Traditionally, cervical screening has relied on cervical cytology, but since 2016, HPV mRNA testing has been integrated to enhance the detection of high-grade lesions (CIN2+) in women with normal cytology. This study, conducted at the Department of Clinical Pathology at UNN from 2016 to 2019, evaluates the impact of HPV mRNA testing on quality assurance, with follow-up adhering to national guidelines through December 2022. Among 98,648 cervical samples analyzed, 61,635 women exhibited normal cytology. Of these, 752 (1.2%) tested positive for HPV mRNA, specifically targeting HPV types 16, 18, and 45. Upon the re-evaluation of the index cytology, 70.7% of these women retained normal cytology findings, while biopsies identified high-grade lesions (CIN2+) in 34% and severe lesions (CIN3+) in 14%. Notably, older women constituted a larger portion of the normal cytology group but a smaller percentage of those testing positive for HPV and exhibiting significant lesions. This underscores the effectiveness of HPV mRNA testing in promptly identifying high-grade lesions, highlighting its potential to significantly reduce cervical cancer incidence through targeted re-evaluation of a small, at-risk population.

Keywords: HPV mRNA testing; cervical screening; Pap smear; cervical cancer; HPV infection dynamics; quality assurance in cervical screening; CIN2+; CIN3+; cervical cytology; public health screening



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1. Introduction

Cervical cancer is the third most common cancer among women aged 25–49 in Norway, accounting for nearly 10% of cases in this group, following only breast cancer and melanoma [1]. It is largely preventable and almost exclusively caused by the human papillomavirus (HPV), which is a sexually transmitted infection. Although 80% of women are estimated to contract HPV at some point—with the majority of infections resolving spontaneously within 24 months [2]—persistent infections can lead to high-grade lesions and eventually cancer [3].

There are over 200 types of HPV, with types 16, 18, and 45 known as the most oncogenic, posing the highest cancer risks to areas like the cervix, penis, vagina, anus, and throat [4,5]. In a study between 2019 and 2021 in Troms and Finnmark, 5.6% of women aged 34–69 tested positive for 14 types of HPV during primary screening [6].

1.1. The Cervical Screening Program

Established in 1995, Norway's national cervical screening program initially recommended that women aged 25–69 undergo cervical cytology every three years [7]. To enhance

cancer prevention efforts, the program has evolved to incorporate HPV testing, which is known for its greater sensitivity compared to cervical cytology [8]. Starting in 2015, a pilot program in certain counties introduced randomized HPV DNA testing every five years for women aged 34–69 (intervention group) alongside the traditional three-year cervical cytology cycle (control group) [9]. In Norway, particularly within the Helse Nord region, strategic and phased implementation of HPV DNA testing in primary cervical screening commenced in 2019, achieving full regional coverage by 2021. Nationwide, the program reached complete implementation for women aged 34–69 by March 2022. Subsequently, in January 2023, women aged 30–33 were integrated into the program, and by July 2023, it was expanded to include those aged 25–29. Now, all women aged 25–69 across Norway are invited to undergo HPV DNA testing as part of the primary screening effort [10].

This comprehensive implementation showcases Norway's excellence in cervical cancer prevention strategies, characterized by the effective use of national registries and a robust social security number system for meticulous individual tracking. These elements, coupled with sufficient resource allocation and rigorous audits, enhance the cost-effectiveness and efficacy of the screening programs, aligning with the Nordic tradition of high standards in public health initiatives.

1.2. HPV Testing as Screening

HPV testing and cervical cytology, both crucial screening methods, have distinct advantages. HPV tests are known for their high sensitivity in detecting high-grade lesions, allowing for longer intervals between screenings—every five years compared to every three years for cervical cytology—without increasing cervical cancer rates [8]. Since HPV is found in nearly 100% of cervical cancer cases, a negative HPV test provides strong reassurance, nearly ruling out the disease and reducing unnecessary follow-ups and healthcare costs [11].

1.3. Types of HPV Tests

HPV DNA test: Commonly used in Norway, the Roche Cobas 4800 identifies DNA from 14 HPV types, with specific emphasis on types 16 and 18, which are most associated with cancer [12]. Its high positivity rate among young adults often leads to many unnecessary follow-ups, making it less suitable for individuals aged 25–33 [13].

HPV mRNA test: This test detects mRNA from the most oncogenic HPV types and is recognized for its high specificity. It provides a more accurate assessment of high cancer risk, making it particularly effective in identifying high-grade lesions in younger women directly linked to cancer risks [14].

Application in quality assurance: For quality assurance in this study, a specific 3-type HPV mRNA test (PreTect SEE) was employed to assess residual material from cytologically normal samples—samples initially judged as NILM (negative for intraepithelial lesion or malignancy) and not previously tested for HPV DNA. This proactive use of the HPV mRNA test aimed to enhance detection sensitivity and potentially identify high-risk cases missed by initial cytology. This approach has significantly improved the detection of high-grade lesions (CIN2+), offering a more targeted and efficient screening process [15].

1.4. Cervical Cytology as a Screening Method

Until 2023, women aged 25–33 in Norway were primarily screened using cervical cytology; however, HPV tests in this age group often produce many false positives due to the high prevalence of the virus but low incidence of cervical cancer. Cervical cytology, interpreted subjectively, carries risks of both false negatives and overlooked high-grade lesions. Despite their 50–70% sensitivity for detecting severe dysplasia (CIN3), significant diagnostic errors occur [16]. In Norway, the incidence of cervical cancer among women under 30 has nearly tripled since the 1950s [17], with over half diagnosed after receiving a normal result on a prior cervical cytology [15].

1.5. Research Question

In a groundbreaking quality assurance initiative, this study at the Department of Clinical Pathology at the University Hospital of North Norway (UNN) evaluates the integration of a 3-type HPV mRNA test with traditional cervical cytology. Conducted from 2016 to 2019, this project targeted women who received normal cytology results but tested positive for HPV, focusing on the re-evaluation of their initial cytology samples. Our analysis meticulously examines the impact of this integration, particularly assessing the proportion of women with positive HPV mRNA tests, adjustments in cytological diagnoses, and the detection of high-grade lesions (CIN2+) during follow-up. The primary goal is to determine how effectively this strategy minimizes misinterpretations of cervical cytology and improves the early identification of women who require further intervention, adhering to national guidelines through December 2022.

2. Results

Of the total number of samples, 76,138 were initially classified as normal. With the phased introduction of the HPV DNA test in 2019, there was a 5% decrease in the number of samples compared to previous years. This affected the categorization of samples as women who tested negative for HPV DNA did not undergo further cervical cytology, impacting the classification under 'normal cytology'. Of the women with normal cervical cytology, 752 (1.2%) tested positive for HPV mRNA. Post the re-evaluation of index cytology, 70.7% of these women continued to show normal cytology, as shown in Table 1. The main objective was to detect high-grade lesions (CIN2+), confirmed through histology, as shown in Table 2 and Figure 1.

Table 1. Revised cytological diagnoses following positive HPV mRNA test results.

Revised Cytological Diagnosis	Number	Percentage (%)
Normal cytology	532	70.7
ASC-US	154	20.5
LSIL	36	4.8
AGUS	10	1.3
ASC-H	16	2.1
HSIL	1	0.1
Cancer	1	0.1
Unsatisfactory	2	0.3
Total	752	100.0

ASC-US: atypical squamous cells of undetermined significance. LSIL: low-grade squamous intraepithelial lesion. AGUS: atypical glandular cells of undetermined significance. ASC-H: atypical squamous cells, which cannot exclude high-grade squamous intraepithelial lesion. HSIL: high-grade squamous intraepithelial lesion.

Table 2. Biopsy findings following positive HPV mRNA test results.

Diagnosis	Number	Percentage (%)
Normal biopsy	87	11.6
CIN1	232	30.9
CIN2	152	20.2
CIN3	70	9.3
ACIS	27	3.6
Cancer	5	0.7
Biopsy not performed	179	23.8
Total	752	100.0

CIN1: cervical intraepithelial neoplasia grade 1. CIN2: cervical intraepithelial neoplasia Grade 2. CIN3: cervical intraepithelial neoplasia grade 3. ACIS: adenocarcinoma in situ.

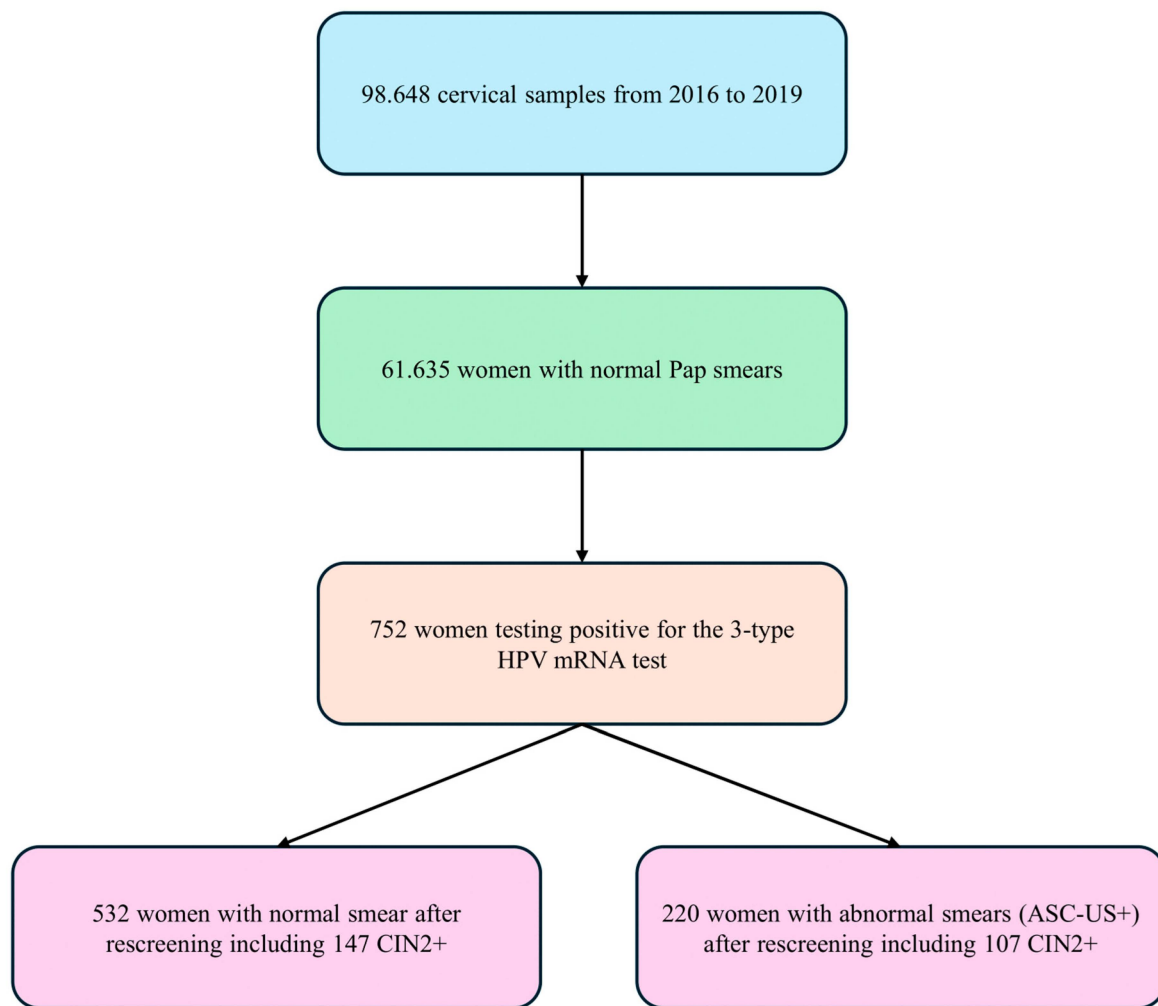


Figure 1. Flowchart of the study population, including HPV mRNA testing, Re-evaluation of the index of cervical cytology, and detection of CIN2+ cases. The HPV mRNA test is a screening tool designed to detect messenger RNA (mRNA) from high-risk types of the human papillomavirus (HPV). For this study, we utilized the PreTect SEE, a 3-type HPV mRNA test specifically targeting the E6/E7 mRNA from HPV types 16, 18, and 45, which are among the most oncogenic and commonly associated with cervical cancer. ASC-US+: atypical squamous cells of undetermined significance or higher (including all categories of abnormalities above ASC-US). CIN2+: cervical intraepithelial neoplasia grade 2 or higher (including CIN2, CIN3, and cancers).

2.1. Detection and Outcomes of High-Grade Cervical Lesions (CIN2+) in HPV mRNA-Positive Cases

Among the 752 women with normal cervical cytology and a positive HPV mRNA test, 254 (33.8%) were diagnosed with CIN2+ on follow-up. The re-evaluation of the initially normal cervical cytology identified abnormalities (ASC-US+) in 220 women (29.3%), with 107 of these women (42.1% of those diagnosed with CIN2+) showing abnormalities upon this first re-evaluation.

Table 3 displays the correlation between revised cytological diagnoses and histologically confirmed CIN2+. Of the 532 women initially diagnosed with normal cytological results, 147 were later found to have CIN2+. Among the 220 with ASC-US+ at re-evaluation, 107 were diagnosed with CIN2+. The sensitivity for detecting CIN2+ at re-evaluation was 42.1%, specificity was 77.3%, PPV was 48.6%, and NPV was 72.4%. The Chi-squared test indicated a statistically significant difference (p -value < 0.001).

Table 3. Comparison of revised cytological diagnoses and detection of CIN2+ cases.

		Biopsy		Total
		CIN1–	CIN2+	
Cytology	Normal	385	147	532
	ASC-US+	113	107	220
	Total	498	254	752

CIN1– Represents histological findings of cervical intraepithelial neoplasia grade 1 (CIN1) and less severe lesions, including normal or benign conditions. CIN2+ represents histological findings of cervical intraepithelial neoplasia grade 2 (CIN2) and more severe lesions, such as cervical intraepithelial neoplasia grade 3 (CIN3), adenocarcinoma in situ (ACIS), and invasive cervical cancer. ASC-US+ represents cytological findings of atypical squamous cells of undetermined significance (ASC-US) and more severe lesions, including low-grade squamous intraepithelial lesions (LSILs) and high-grade squamous intraepithelial lesions (HSILs).

2.2. Detection and Outcomes of Severe Cervical Lesions (CIN3+) in HPV mRNA-Positive Cases

Of the same group of 752 women, 102 (13.6%) were diagnosed with CIN3+ upon later follow-up. Nearly half of these cases (48.0% or 49 women) were only identified as abnormal (ASC-US+) during the re-evaluation of their initial cervical cytology.

Table 4 outlines the relationship between revised cytological diagnoses and histologically confirmed CIN3+. Out of the 532 women with initially normal results, 53 were diagnosed with CIN3+. Among those with ASC-US+ at re-evaluation, 49 were found to have CIN3+, achieving a sensitivity of 48.0%, specificity of 73.7%, PPV of 22.3%, and NPV of 90.0%. The Chi-squared test also confirmed the significance of these findings (*p*-value < 0.001).

Table 4. Comparison of revised cytological diagnoses and detection of CIN3+ cases.

		Biopsy		Total
		CIN2–	CIN3+	
Cytology	Normal	479	53	532
	ASC-US+	171	49	220
	Total	650	102	752

CIN2– represents histological findings of cervical intraepithelial neoplasia grade 2 (CIN2) and less severe lesions, including cervical intraepithelial neoplasia grade 1 (CIN1) and normal or benign conditions. CIN3+ represents histological findings of cervical intraepithelial neoplasia grade 3 (CIN3) and more severe lesions, such as adenocarcinoma in situ (ACIS) and invasive cervical cancer. ASC-US+ represents cytological findings of atypical squamous cells of undetermined significance (ASC-US) and more severe lesions, including low-grade squamous intraepithelial lesions (LSILs) and high-grade squamous intraepithelial lesions (HSILs).

2.3. Detection and Outcomes of Cancer in HPV mRNA-Positive Cases

In our study, 752 women had initially normal cytology results but tested positive for HPV mRNA. During the follow-up period, cervical cancer was detected in five of these women, which corresponds to a prevalence rate of 0.7%. Notably, four of these five cancer cases had their initial ‘normal’ cytology results revised to ‘ASC-US+’ upon re-evaluation prompted by the positive HPV mRNA test result. This re-evaluation was part of our quality assurance process using the 3-type HPV mRNA test, which specifically targets the most oncogenic HPV types and is sensitive to changes in cellular activity that may not be captured in primary cytology screenings.

This subset of women with ‘ASC-US+’-revised diagnoses accounted for the majority of the diagnosed cancer cases in the study, underscoring the importance of HPV mRNA testing as a supplementary diagnostic tool. It highlights that without the HPV mRNA test, these cases might have progressed undetected until the next scheduled screening, potentially allowing the cancer to develop further.

2.4. Age-Related Distribution and Outcomes of HPV and Cervical Lesions

In this study, women aged 34–69 formed the largest cohort, comprising over 70% of participants with normal cervical cytology. Yet, this group accounted for only 43% of positive HPV mRNA tests and 34% of high-grade lesions (CIN2+), as shown in Figure 2. Conversely, women aged 25–33, who made up just 21% of those with normal cytology, represented 39% of those with positive HPV mRNA tests and 46% of CIN2+ cases, as illustrated in Figure 2. Notably, women under 25 showed the highest risk, with 3.9% testing positive for HPV and 1.5% for CIN2+ despite having normal cytology, as depicted in Figure 3.

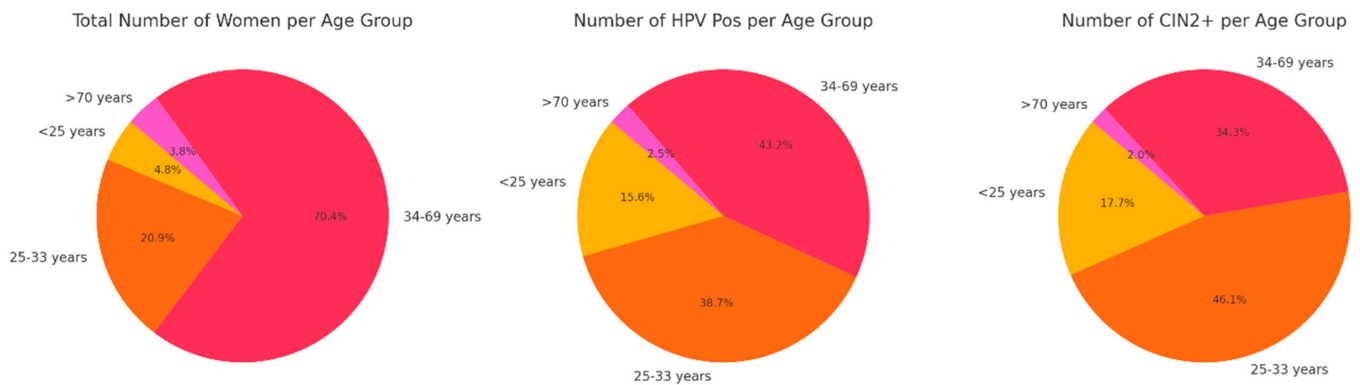


Figure 2. Distribution of HPV-positive women and detection of CIN2+ cases by age group. CIN2+ represents histological findings of cervical intraepithelial neoplasia grade 2 (CIN2) and more severe lesions, such as cervical intraepithelial neoplasia grade 3 (CIN3), adenocarcinoma in situ (ACIS), and invasive cervical cancer.

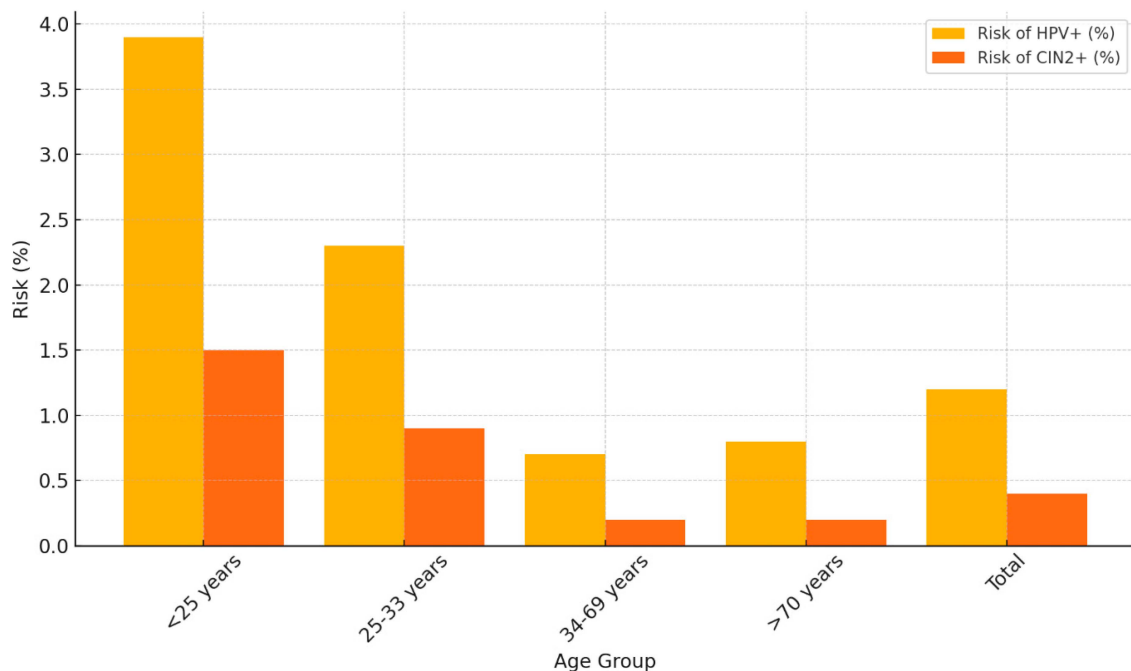


Figure 3. Risk of being HPV-positive and developing CIN2+ by age group. HPV+: indicates a positive result from a 3-type HPV mRNA test (PreTect SEE) that detected E6/E7 mRNA from high-risk human papillomavirus types 16, 18, and 45, suggesting an increased risk of cervical cancer development. CIN2+ represents histological findings of cervical intraepithelial neoplasia grade 2 (CIN2) and more severe lesions, including cervical intraepithelial neoplasia grade 3 (CIN3), adenocarcinoma in situ (ACIS), and invasive cervical cancer, indicating progressively higher risks of cancer progression.

Table 5 displays the distribution and proportions of women in each age group, highlighting the disparity in HPV positivity and CIN2+ risk. These findings demonstrate that younger women, particularly those aged 25–33, have a higher incidence of positive HPV mRNA tests and CIN2+, underscoring the need for focused screening and follow-up in these demographics.

Table 5. Distribution of HPV positivity and risk of CIN2+ by age group among women with normal cervical cytology.

Age Group	Number	(%)	HPV Pos	(%)	Risk (%)	CIN2+	(%)	Risk (%)
<25 years	2973	4.8	117	15.6	3.9	45	17.7	1.5
25–33 years	12,901	20.9	291	38.7	2.3	117	46.1	0.9
34–69 years	43,399	70.4	325	43.2	0.7	87	34.3	0.2
>70 years	2362	3.8	19	2.5	0.8	5	2.0	0.2
Total	61,635	100.0	752	100.0	1.2	254	100.0	0.4

CIN2+ represents histological findings of cervical intraepithelial neoplasia grade 2 (CIN2) and more severe lesions, including cervical intraepithelial neoplasia grade 3 (CIN3), adenocarcinoma in situ (ACIS), and invasive cervical cancer, indicating progressively higher risks of cancer progression.

3. Materials and Methods

The Department of Clinical Pathology at the University Hospital of North Norway (UNN) analyzes approximately 24,000 cervical samples annually from Troms and Finnmark. Data for this study were extracted from SymPathy [18], the clinic’s database system, and reorganized in IBM SPSS Statistics for Windows, Version 29.0.1.0 for analysis [19]. Between 2016 and 2019, a total of 98,648 samples were collected. This study analyzed the initial samples from 61,635 women with normal cervical cytology, aged 25 to 69 years, aligning with the Cervical Screening Program’s recommendations. To ensure consistency, only the first sample provided by each participant during the study period was included; for example, if a woman submitted samples in both 2016 and 2019, only the 2016 sample was analyzed. HPV mRNA testing utilized the 3-type HPV E6/E7 mRNA test (PreTect SEE, PreTect AS, Klokkarstua, Norway), targeting genotypes 16, 18, and 45. The follow-up was rigorously adhered to in line with national guidelines, continuing until December 2022. We utilized histologically confirmed CIN2+ as the study endpoint. All biopsy specimens were reviewed by two experienced pathologists to ensure diagnostic accuracy. In cases where diagnosis proved challenging, P16(INK4a) immunostaining (Roche mtm laboratories AG, Heidelberg, Germany) was utilized as an adjunct to support and clarify histological findings. Any discrepancies between the initial biopsy results and treatment-derived histology were resolved by adopting a more severe diagnosis as the definitive study endpoint.

3.1. Study Design

This cohort study evaluates the impact of quality assurance measures on detecting high-grade lesions (CIN2+) in normal cervical cytology using the HPV mRNA test. Women typically screened every three years can benefit from earlier detection. Those testing positive for HPV receive a more intensive follow-up per national guidelines, enhancing the early detection and treatment of potential cancers.

Initial HPV mRNA testing: residual material from the initial liquid-based cytology (LBC), which initially showed normal results, was tested for HPV mRNA within 2–3 weeks.

Re-evaluation process: if HPV mRNA results were positive for types 16, 18, or 45, the original cytology sample was re-evaluated by a different cytologist and double-checked by a pathologist.

Follow-up procedures: After initial HPV mRNA testing, the following took place:

- If re-evaluation showed normal cytology, women were recommended to have a repeat cytology and HPV test after 12 months.

- If re-evaluation showed low-grade changes (ASC-US/LSIL),
 - For HPV types 16/18, colposcopy and biopsy were recommended within 1–3 months.
 - For HPV type 45, a follow-up with repeat cytology and HPV testing after 12 months was recommended.
- If re-evaluation showed high-grade lesions (ASC-H/HSIL), colposcopy and biopsy were recommended within 1–3 months.

This protocol ensured that all women received appropriate and timely follow-up based on the specific risk indicated by their re-evaluated cytology and HPV mRNA test results, aligning with the three-year national screening interval.

3.2. Statistical Methods

Data from cross-tabulations 3, 4, and 5 were analyzed using SPSS, employing Chi-squared tests to assess statistical significance, with a p -value < 0.05 indicating a significant difference.

3.3. Ethical Approval

The Regional Committee for Medical and Health Research Ethics (REK Nord) approved this study as a quality assurance project in laboratory work (2013/497/REK Nord, 2013/927/REK Nord, and 230825 REK Nord). The use of anonymized data eliminated the need for further approval.

4. Discussion

This study evaluated the effectiveness of employing a 3-type HPV mRNA test alongside routine cervical cytology to identify treatable high-grade lesions (CIN2+) and thereby reduce cancer risk. Within the cohort of 61,635 women with initially normal cervical cytology, 1.2% (752 women) tested positive for HPV mRNA. Upon re-evaluation, 70.7% of these women continued to present normal cytology results, while the remaining participants were found to have varying degrees of cytological abnormalities, primarily ASC-US and LSIL.

Notably, the observed 20.5% ASC-US rate among the HPV mRNA-positive subgroup significantly exceeded the typical expectations for a general screening population. However, this rate pertains exclusively to a high-risk subset—those who initially tested negative for cytological abnormalities but were subsequently identified by HPV mRNA testing as having potential lesions. Thus, while the ASC-US percentage might seem high, it reflects a focused re-evaluation of a select group deemed at increased risk due to their HPV status. This targeted approach resulted in a minor absolute increase of only 0.25% in ASC-US rates across the entire cohort of 98,648 samples, underscoring the precision of our screening strategy in enhancing the detection of potential high-risk cases without considerably increasing the overall burden of follow-up procedures. The impact of this refined detection method on our ability to prevent cervical cancer in women initially diagnosed with normal cytology is profound, and we have revised the manuscript to clearly communicate these dynamics and their implications.

Of the women testing positive for HPV mRNA, 76.2% underwent subsequent colposcopy and biopsy. A third of these women (33.8%) were diagnosed with CIN2+, with 107 having a revised diagnosis of ASC-US+. The re-evaluation sensitivity for detecting CIN2+ was 42.1%, indicating that continuous follow-up is crucial for women with positive HPV tests but normal cervical cytology due to the persistently increased risk of CIN2+.

In our analysis, approximately one-fourth of the cases did not proceed to biopsy following colposcopy, which initially may seem concerning given the potential for missing high-grade lesions. However, these cases were managed in strict accordance with Norwegian national guidelines, which dictate that a biopsy should always be performed if colposcopy is indicated unless the colposcopic examination reveals no visible lesions. In such instances, our clinicians performed blind biopsies from all four quadrants of the transformation zone and conducted an endocervical curettage (ECC) as per the standard

protocol. Additionally, for cases where biopsies were not conducted due to the absence of visible lesions or in the context of conservative management for transient infections, follow-up was meticulously ensured with repeat cytology and HPV testing. This approach aligns with the need to balance intervention and observation, particularly for younger women or those with transient HPV infections.

Moreover, our study addresses the significant challenges posed by type 3 transformation zones, where high-grade lesions may be hidden and not readily visible during colposcopy. For women in this category with persistent high-risk HPV indications or escalating cytological abnormalities and negative initial biopsy results, our protocol mandates further rigorous follow-up, including repeat cytology, HPV testing, and a potential diagnostic excisional procedure like conization (LEEP) if abnormalities persist. This layered approach ensures that no potential high-grade lesions are overlooked, particularly in complex clinical scenarios involving type 3 transformation zones, thereby reinforcing the effectiveness and safety of our cervical cancer screening and management strategy. These measures are crucial in mitigating the risk of progression to invasive cancer, thereby enhancing patient outcomes.

The positive predictive value (PPVs) for identifying CIN2+ in re-evaluated samples initially assessed as normal was 48.6%. This shows that nearly half of the women with a revised abnormal cytological finding (ASC-US+) and a positive HPV test had high-grade lesions, significantly highlighting the increased CIN2+ risk in this subgroup compared to those with normal findings at re-evaluation, who had a risk of 27.6%.

A high-positive predictive value (PPV) is crucial for minimizing unnecessary biopsies among women with abnormal screening results but at low risk of cervical cancer [20]. Among the 532 women who tested positive for HPV mRNA but had normal cytology, 147 (27.6%) were later diagnosed with CIN2+. Given that the current threshold for colposcopy and biopsy referrals is 20%, our data robustly support the recommendation for direct colposcopy and biopsy in all women testing positive for HPV mRNA, irrespective of their re-evaluated cytology results [21]. This proactive approach is justified by the high-risk nature of positive HPV mRNA results, which indicate active viral transcription, potentially leading to significant lesions or cancer.

This strategy could streamline the screening process by eliminating the need for additional cytological re-evaluation in those who test positive for HPV mRNA, thus expediting the intervention for high-risk cases. Furthermore, while emerging biomarkers like methylation and dual staining (p16/Ki67) show promise and are being integrated into practice in some regions, the immediate applicability and established reliability of HPV mRNA tests render them a particularly valuable tool in the current landscape of cervical cancer prevention, especially until such a time as methylation markers are more fully validated and integrated into national guidelines [6].

Among women with normal cervical cytology and a positive HPV mRNA test, 13.6% (102 out of 752) were diagnosed with CIN3+ at follow-up. Notably, 48.0% (49 out of 102) of these CIN3+ cases, initially missed, were detected as abnormal (ASC-US+) upon the re-evaluation of initial cytology. This underscores the efficacy of quality assurance measures in identifying severe dysplasia. Additionally, the risk for CIN3+ among women who are HPV mRNA-positive with normal cytology upon re-evaluation was approximately 10% (53 out of 532), highlighting the need for thorough follow-up in this demographic.

Interestingly, our findings indicated a lower CIN3+ risk (13.6%) compared to the 28.6% reported by Al-Shibli et al., potentially due to the differences in age groups, follow-up durations, and diagnostic methodologies between UNN and Bodø [22]. Al-Shibli et al.'s study also underscored the increased sensitivity of HPV mRNA testing in screening, which is particularly effective at identifying women with normal cytology but at low risk of cervical cancer, thereby emphasizing the importance of molecular diagnostics in improving early detection [22].

Similarly, Westre et al. demonstrated that including HPV mRNA tests in screening programs increases the detection of CIN3 by 17–18% among women under 40 with normal

cytology [15]. This supports the assertion that HPV-based tests, which are more objective and sensitive than cervical cytology, are essential for accurately identifying women at increased risk of cancer.

In 2023, the Cervical Screening Program implemented a 14-type HPV DNA test as the standard testing method to improve the detection of potentially cancerous HPV infections [23]. While this broad-spectrum approach enhanced the capability to intercept and treat early-stage diseases, it also raised concerns about the over-identification of low-risk HPV infections in women, potentially leading to unnecessary interventions for conditions likely to resolve spontaneously [24]. This is particularly significant in younger women aged 25–30, where the high prevalence of HPV does not always correlate with an increased risk of cervical cancer. Recent adaptations in screening guidelines recommend a more tailored approach, potentially restricting HPV testing to the types included in the nonvalent vaccine to optimize the balance of harms and benefits. Studies suggest that focusing on HPV types 16, 18, 31, 33, 45, and 52 could significantly reduce unnecessary follow-ups without compromising the detection of serious pathologies [25].

The variability in cytological screening sensitivity is considerable [26]; as per the ATHENA study, it ranged from 42.0% to 73.0% across different laboratories [27]. For our cohort, 42.1% of the women diagnosed with CIN2+ upon follow-up had abnormal cytology upon the re-evaluation of the initial cytology sample despite initially normal evaluations, highlighting the increased sensitivity afforded by incorporating HPV mRNA testing.

Additionally, 33.8% of women with positive HPV mRNA tests were identified with CIN2+. In 2022, among the 257 Norwegian women aged 25 to 69 diagnosed with cervical cancer, 45 had received a normal cervical cytology result within the previous 3.5 years [28]. This statistic underscores the limitations of using cervical cytology within the current three-year screening interval, particularly given that cervical cancer can develop from severe dysplasia (CIN3+) to invasive stages over a much longer period, typically 10–20 years. These findings emphasize the urgent need for more effective early detection methods that can identify precancerous changes more reliably and earlier on, potentially necessitating adjustments to typical screening intervals.

Significantly, women aged 25–33 years constituted 39% of those with a positive HPV mRNA test and 46% with CIN2+, indicating a higher disease burden in younger demographics. Women under 25 faced the greatest risk, with 3.9% testing positive for HPV and 1.5% for CIN2+, far exceeding the risk levels in women aged 34–69 (0.7% for HPV, 0.2% for CIN2+). These data emphasize the importance of targeted preventive measures for younger women, especially given the long-term timeline for the full protective effect of the HPV vaccine introduced into the national immunization program in 2009.

Even though HPV infections and minor cytological lesions can resolve spontaneously, especially among younger individuals [13], screening programs with enhanced sensitivity are crucial. They help to ensure that high-grade lesions are not overlooked, thereby extending the opportunity for necessary follow-up and intervention [22].

Cervical cancer is theoretically close to eradicable with screening programs that effectively identify high-risk individuals early enough for timely treatment [29]. In our study, 1.2% of 61,635 women with normal cervical cytology (752 women) tested positive for HPV mRNA. This is comparable to the 2.6% reported by Al-Shibli et al. [22] and 1.9% by Westre et al. [15], indicating a low percentage of samples needing re-evaluation when using the HPV mRNA test for quality assurance. Given that this test can enhance screening sensitivity by about 17–18%, the benefits significantly outweigh the slight increase in workload [15].

The population-based design of our study, encompassing all women from the Troms and Finnmark counties attending cervical cancer screening, significantly bolsters the robustness of our findings. These women were co-tested using cervical cytology and the 3-type HPV mRNA test, with follow-ups conducted according to national guidelines. This comprehensive approach not only validates the effectiveness of HPV mRNA testing within our screening context but also suggests its broader applicability to similar cytology-based screening programs worldwide.

Furthermore, our study underscores the potential of HPV mRNA testing as a more specific tool compared to HPV DNA testing, particularly among younger women. In this demographic, the high prevalence of transient infections often leads to elevated false-positive rates with DNA testing. The enhanced specificity of HPV mRNA tests is crucial for reducing unnecessary follow-ups and interventions, thereby making it an invaluable asset in age-stratified screening protocols.

A study from Poland involving 125 women tested with a 5-type HPV mRNA assay (NucliSENS EasyQ® HPV v1.1 by bioMérieux, Marcy-l'Étoile, France) reported a sensitivity for CIN2+ of 86.1% (31/36) [30]. Additionally, a Chinese study of 1387 patients with ASC-US cytology who tested positive for HPV E6/E7 mRNA using the Hologic APTIMA assay demonstrated that 62.2% (219/352) of women with histologically confirmed HSIL+ had HPV types 16, 18, or 45, and this included 67.2% (43/64) of women diagnosed with cervical cancer [31].

In Norway, the HPV vaccination was introduced into the childhood immunization program in 2009 for 12-year-old girls and extended to include boys in 2018. Currently, vaccination coverage among 12-year-olds stands at approximately 90%. The first cohort of vaccinated girls reached the age of 25 in 2022, which is the starting age for participation in the national cervical cancer screening program, which covers women aged 25 to 69. According to data from the Norwegian Cancer Registry, only one woman who was vaccinated in 2009 or 2010 has been diagnosed with cervical cancer in 2022 or 2023 [32]. Adult vaccination is almost nonexistent in Norway. In our study, among the five cases of cervical cancer detected in women with initially normal cytology and positive HPV mRNA tests, four were identified following the re-evaluation of initial normal cytology results and one during the follow-up of women with positive HPV mRNA tests but without cytological abnormalities. None of the women diagnosed with cervical cancer in our study had received the HPV vaccine. This context underscores the significance of HPV vaccination in reducing cervical cancer incidence and highlights the impact of our findings in a largely unvaccinated adult population.

However, a limitation of this study is its exclusion of women who did not test positive for HPV mRNA but might still harbor high-grade lesions (CIN2+) detectable by traditional cervical cytology. The follow-up of women with a positive HPV mRNA test was based solely on cytological findings, potentially affecting the detection rate of CIN2+ in those with normal cytology upon re-evaluation. An approach that involves immediate colposcopy and biopsy for all women testing positive for HPV mRNA could uncover more CIN2+ cases but might also lead to overtreatment.

For a more comprehensive understanding, a comparative analysis with a control group undergoing only traditional cervical cytology would be beneficial. Such a study could clarify the relative effectiveness of HPV mRNA testing. With the recent introduction of the HPV DNA test for primary screening in Norway for women aged 25–33, comparing this with co-testing involving cervical cytology and the 3-type HPV mRNA test would be insightful, especially concerning the risk of unnecessary follow-ups and overtreatments.

5. Conclusions

This study assessed the impact of the 3-type HPV mRNA test on detecting high-grade lesions (CIN2+) in normal cervical cytology among women attending cervical cancer screening in the Troms and Finnmark counties. We found that 1.2% of women with normal cytology tested positive for HPV mRNA, with 33.8% of these later diagnosed with CIN2+ and 13.6% diagnosed with CIN3+. Notably, 48% of the CIN3+ cases were detected upon re-evaluation, highlighting the role of HPV mRNA testing in enhancing the detection of severe dysplasia and potentially reducing cervical cancer incidence by facilitating earlier treatment.

Future research should investigate the long-term outcomes of women who test positive for HPV mRNA, focusing on the progression of CIN2+ conditions and assessing the cost-

effectiveness and psychosocial impacts of incorporating HPV mRNA testing into routine cervical cancer screening programs.

In summary, HPV mRNA testing significantly augments traditional cytological screening, improving the early detection and management of cervical cancer. This supports the need for its broader integration into screening strategies.

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Informed Consent Statement: This register-based, retrospective study was derived from a quality assurance project involving anonymized data. Consequently, the requirement for informed consent was waived by REK Nord, based on the protocols cited in the ethics approval.

Data Availability Statement: The raw data supporting the conclusions of this article are derived from an anonymized dataset used under the approval for a quality assurance project by the Regional Committee for Medical and Health Research Ethics (REK Nord). Given the nature of this research and the ethical restrictions concerning the privacy of individuals, the raw data are not publicly available. The data are available from the corresponding author upon reasonable request.

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