



Case Report

High-Risk HPV Positivity With NILM Cytology: The Added Value of HPV Genotyping in a Case Report

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Abstract

Background: High-risk human papillomavirus (HR-HPV) positivity with negative for intraepithelial lesion or malignancy (NILM) cytology represents a discordant cervical cancer screening result in which HPV DNA is detected despite the absence of cytological evidence of an intraepithelial lesion or malignancy. HPV genotyping and molecular triage approaches have been evaluated to further characterise risk among HPV-positive women. **Case Presentation:** A cervical cytology specimen obtained during cervical cancer screening was processed using liquid-based cytology with the ThinPrep® system and Papanicolaou staining. Microscopic examination demonstrated a satisfactory specimen with adequate representation of squamous epithelial cells and no cytological evidence of intraepithelial lesion or malignancy. High-risk HPV testing was performed using polymerase chain reaction with the Xpert® HPV HR assay on the GeneXpert® Dx System. HPV16 and HPV18/45 were negative, as were the HPV31/35/33/52/58 and HPV51/59 genotype groups. HPV DNA was detected within the HPV39/68/56/66 genotype group. Sample adequacy and probe check controls passed, and the test was completed successfully without an error. **Integrated Interpretation:** The case demonstrates an HR-HPV-positive/NILM cytological pattern. The positive molecular result indicates detection of high-risk HPV DNA but does not establish the presence of cervical intraepithelial neoplasia or a precancerous lesion. Because the Xpert® HPV HR assay reports the positive result as a genotype group, the specific genotype responsible for the positive result cannot be determined from this test alone. **Conclusion:** This case illustrates the added information provided by HPV genotype-group analysis in an HR-HPV-positive/NILM screening result. The findings support risk-based clinical follow-up according to applicable cervical cancer screening guidelines rather than a diagnosis of precancerous disease.

Keywords: Pap test, HR HPV Test, Cervical Cytology, HPV Serotype, NILM.

Introduction

High-risk human papillomavirus (HR-HPV) positivity with negative for intraepithelial lesion or malignancy (NILM) cytology represents an important setting for cervical cancer screening triage. A recent study specifically evaluated HPV-positive women with NILM, ASC-US, and LSIL cytology to determine whether GYPC methylation and HPV genotyping could improve the identification of women with cervical intraepithelial neoplasia grade 2 or worse (CIN2+).¹ HPV genotyping can provide additional information for risk stratification among HPV-positive women. In the Italian NTCC2 study, HPV genotypes were divided into 3 groups according to their risk of CIN3+, and HPV-positive women were followed for 24 months or until HPV clearance.² Among HPV-positive women with negative cytology or p16/Ki67 dual staining, combining extended HPV genotyping with the triage result identified

ntified groups with different risks of CIN3+.² The study identified a group with a 24-month CIN3+ risk of 0.4%, consisting of women with negative cytology or dual-stain results and specific HPV genotype categories, who could be considered for longer-interval retesting.² p16/Ki67 dual-stain cytology has also been evaluated as a triage method for HPV-positive women. In the IMPACT trial, 4927 HPV-positive women with valid dual-stain results were included in the analysis.³ Dual-stain sensitivity for CIN2+ and CIN3+ was 91.2% and 91.9%, respectively, among HPV16/18-positive women, and 83.0% and 86.0%, respectively, among women infected with 12 other HPV genotypes.³ The risk of CIN2+ among HPV-positive women with a negative dual-stain result was 3.6%, which was lower than the corresponding risks among women with negative cytology-based triage strategies.³ DNA methylation has also been evaluated as





a triage biomarker among HR-HPV-positive women. In a study of HPV-positive women with NILM, ASC-US, and LSIL cytology, GYPC methylation distinguished women with CIN2+ from those with less than CIN2, with an area under the receiver operating characteristic curve of 0.828.¹ The CIN2+ risk was 36.2% among women with positive GYPC methylation and 2.3% among women with negative GYPC methylation.¹ Combining HPV16/18 genotyping with GYPC methylation resulted in different absolute CIN2+ risks according to the combined test results.¹ The colposcopy referral rate for the HPV16/18-or-GYPC-methylation strategy was 35.6%, compared with 79.2% for the HPV16/18-or-ASC-US+ strategy, while the reported sensitivities were similar.¹ ZNF582 methylation has also been evaluated in HR-HPV-positive women as a biomarker for detecting high-grade cervical lesions. In a study involving 199 HR-HPV-positive women, ZNF582 methylation was compared with liquid-based cytology and p16/Ki67 dual-stain cytology using cervical pathology as the reference.⁴ The AUC for distinguishing less than CIN3 from CIN3+ was 0.797.⁴ ZNF582 methylation showed a specificity of 88.4% for CIN3+, compared with 28.6% for cytology and 43.5% for p16/Ki67 dual-stain cytology.⁴ The combination of HPV16/18 genotyping with ZNF582 methylation was also evaluated, and the HPV16/18-or-ZNF582-methylation strategy showed a specificity of 63.3%.⁴ MicroRNAs have additionally been investigated using liquid-based cytology specimens from HPV-positive women. A 2026 study evaluated 6 microRNAs in residual cellular material from liquid-based cytology specimens and compared their expression among women with NILM, CIN II, CIN III, and cervical cancer.⁵ Stage-dependent changes in microRNA expression were observed across these disease categories.⁵ In particular, miR-16-5p, miR-20b-5p, and miR-155-5p demonstrated high diagnostic accuracy for distinguishing NILM from high-grade lesions and invasive cervical cancer.⁵ The study also reported progressive remodeling of

microRNA co-expression networks, with the most pronounced changes occurring during the transition from CIN II to CIN III.⁵ The findings from these studies demonstrate that HPV genotyping, cytological triage, p16/Ki67 dual staining, DNA methylation, and microRNA analysis have each been evaluated for risk assessment among HPV-positive women.¹⁻⁵ Studies combining HPV genotype information with cytological or molecular triage have also demonstrated differences in the risk of CIN2+ or CIN3+ according to the combined test results.^{1,2,4} Therefore, evaluation of cytology, HPV genotype, and biomarker-based information in HR-HPV-positive women with NILM cytology provides a basis for investigating an integrated approach to risk stratification of cervical intraepithelial neoplasia.

Case Presentation

Clinical and Laboratory Presentation

A cervical cytology specimen was obtained from a 34-year-old woman undergoing cervical cancer screening. The specimen was processed using liquid-based cytology with the ThinPrep® system, followed by Papanicolaou staining for cytomorphological evaluation.

Microscopic examination demonstrated a satisfactory specimen with adequate representation of squamous epithelial cells and no cytological evidence of an intraepithelial lesion or malignancy. Figure 1

The squamous epithelial cells showed preserved nuclear-to-cytoplasmic ratios, smooth and regular nuclear contours, finely granular chromatin, and inconspicuous nucleoli. No significant nuclear enlargement, hyperchromasia, irregular nuclear membrane, increased nuclear-to-cytoplasmic ratio, or abnormal mitotic activity was identified. No cytomorphological features diagnostic of ASC-US, LSIL, HSIL, or invasive malignancy were observed. The cytological interpretation was therefore according to Bethesda Category Negative for Intraepithelial Lesion or Malignancy (NILM).

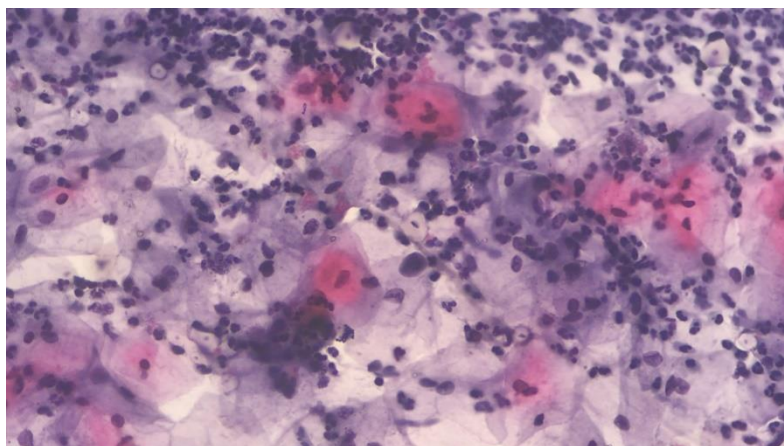


Figure 1: Illustrates a loose cluster of Superficial and intermediate squamous cells with inflammatory cells (Pap stain, x40).



High-Risk HPV Testing

High-risk HPV testing was performed using polymerase chain reaction with the Xpert® HPV HR assay on the GeneXpert® Dx System. The assay demonstrated high-risk HPV positivity with the following results: HPV 16, 18, 45, 31, 35, 33, 52, 58, 51, and 59 were negative, while HPV 39/68/56/66 were positive; the Sample Adequacy Control and Probe Check Control passed, and the test status was completed successfully. No Error status in the procedure.

The molecular findings indicated the detection of HPV DNA within the HPV 39/68/56/66 genotype group targeted by the assay. HPV 16, HPV 18/45, HPV 31/35/33/52/58, and HPV 51/59 were not detected.

Because the assay reports results for genotype groups rather than individual genotypes within the HPV 39/68/56/66 channel, the specific genotype responsible for the positive result cannot be determined from this test alone.

Integrated Interpretation

This case demonstrates a discordant screening result consisting of NILM cytology and a positive high-risk HPV test. The cytological findings indicate that no intraepithelial lesion or malignancy was identified in the examined specimen at the time of testing. The positive HPV result indicates detection of high-risk HPV DNA, but it does not by itself establish the presence of cervical intraepithelial neoplasia or a precancerous lesion.

The absence of cytological abnormalities may reflect the absence of detectable morphological changes at the time of sampling, although cytology and HPV testing assess different biological features. High-risk HPV infection is common and may be transient; therefore, the clinical significance of this result depends on factors such as persistence of infection, the patient's age and screening history, prior abnormalities, and applicable clinical management guidelines.

Ethical Approval

Ethical approval for this work was obtained from Saray Salam Diagnostic Centre.

Molecular Testing

HPV detection and genotype-group analysis were performed using polymerase chain reaction with the Xpert® HPV HR assay on the GeneXpert® Dx System. At Reference Medical Laboratory Benghazi R.M.L.B

Discussion

The present case was negative for intraepithelial lesion or malignancy (NILM) cytology with a positive high-risk human papillomavirus (HR-HPV) result. HPV16 and HPV18/45 were not detected, whereas the Xpert HPV HR assay detected HPV DNA within the HPV39/68/56/66 genotype group. Recent studies have specifically evaluated HPV-positive women with normal cytology and demonstrated differences in cervical intraepithelial neoplasia risk according to HPV genotype[6,7]. A multicenter prospective cohort study

evaluated 8401 women with NILM cytology using a 14-type HPV real-time PCR assay with HPV16/18 genotyping[6]. Among 6679 women who completed follow-up, the 3-year cumulative CIN2+ risk was 20.9% for HPV16/18-positive women, 6.6% for women positive for other HPV types, and 0.1% for HPV-negative women[6]. HPV positivity showed a sensitivity of 92.3% and specificity of 89.6% for CIN2+, while HPV16/18 positivity showed a sensitivity of 41.0% and specificity of 98.2%[6]. The authors reported that HPV16/18 genotyping provided risk stratification among women with NILM cytology[6]. Extended HPV genotyping has also been evaluated according to genotype-specific risk. In the baseline phase of the Onclarity trial, the $\geq$ CIN3 risk among women with NILM cytology was 2.1% for HPV33/58/52 and 0.6% for HPV45/35/39/68/51/56/59/66[7]. The study reported that extended genotyping generated risk strata according to HPV genotype and cytology and concluded that extended genotyping provided flexible risk stratification for colposcopy thresholds[7]. The HPV39 and HPV68 detected within the genotype group in the present case are therefore included among the extended genotype categories evaluated in this study[7]. The Italian NTCC2 study similarly evaluated extended HPV genotyping combined with cytology or p16/Ki67 dual staining[8]. HPV genotypes were classified into three groups according to CIN3+ risk[8]. Among women with negative cytology or negative dual-stain results, the combined genotype and triage results identified groups with different 24-month CIN3+ risks.³ The study reported a 24-month CIN3+ risk of 0.4% in a group including women with negative cytology or dual-stain results and HPV56/59/66 or HPV35/39/68 infection[8]. The clinical management of extended genotype results has been addressed by the Enduring Consensus Cervical Cancer Screening and Management Guidelines[9]. The guideline recommends colposcopy for positive HPV16 or HPV18 results[9]. For women positive for HPV45, HPV33/58, HPV31, HPV52, HPV35/39/68, or HPV51 but negative for HPV16 and HPV18, cytology or dual-stain testing is recommended for triage[9]. For primary HPV screening, women positive only for HPV56/59/66 are recommended to undergo repeat HPV testing at 1 year[11]. For contesting, women positive for HPV56/59/66 with NILM, ASC-US, or LSIL cytology are also recommended to return in 1 year[9]. The Xpert HPV HR assay is particularly relevant to the present case because it provides extended HPV genotyping through genotype-group channels. A 2026 study evaluated Xpert HPV extended genotyping against histology in 1063 women aged 25 to 65 years[10]. The study defined the Top14 group as HPV16, 18, 45, 31, 33, 35, 52, 58, 51, 59, 39, 56, 66, and 68[10]. The authors evaluated Top3, Top8, and Top14 genotype thresholds and reported that screening for all 14 high-risk HPV types provided the highest sensitivity but required triage,



whereas the Top8 threshold provided the best balance between sensitivity and specificity in the investigated test-and-treat setting[10].The laboratory interpretation of the present Xpert result is therefore based on a genotype group rather than identification of one individual HPV type. The Xpert study specifically describes HPV39, HPV56, HPV66, and HPV68 as separate members of the Top14 extended genotype group[10].Consequently, the positive HPV39/68/56/66 channel represents detection within this group, rather than identification of one specific genotype from the group[10].A 2025 systematic review and meta-analysis evaluated point-of-care HPV tests and reported pooled performance of the Xpert HPV assay[11].For physician-collected cervical samples, the pooled sensitivity and specificity of Xpert HPV for CIN2+ were 92.3% and 53.3%, respectively[11].For self-collected vaginal samples, the corresponding pooled sensitivity and specificity were 91.5% and 56.5%.[11].The study reported that Xpert HPV demonstrated good sensitivity and specificity as a point-of-care cervical cancer screening method[11].Recent laboratory guidance has also addressed the use of extended HPV genotyping in HPV-positive screening. Goulart et al. described extended HPV genotyping and dual-stain testing as triage approaches for primary HPV screen-positive cases[12].The authors emphasised that HPV-positive women require triage and that the triage method should balance sensitivity for precancer with the number of colposcopies required[12].The guidance also noted that no screening or triage test can completely exclude disease in a screen-positive patient[12].Molecular biomarkers have additionally been investigated for triage of HPV-positive women with normal or minimal cytological findings. Mao et al. evaluated GYPC methylation in HPV-positive women with NILM, ASC-US, and LSIL cytology[13].GYPC methylation distinguished less than CIN2 from CIN2+ with an AUC of 0.828[13].The CIN2+ risk was 36.2% among women with positive GYPC methylation and 2.3% among women with negative GYPC methylation[13].The combination of HPV16/18 genotyping with GYPC methylation produced different absolute CIN2+ risks according to the combined test results, while the HPV16/18-or-GYPC-methylation strategy had a colposcopy referral rate of 35.6% compared with 79.2% for HPV16/18-or-ASC-US+ triage[13].A separate study evaluated GYPC methylation using residual exfoliated cervical cells from liquid-based cervical samples in HR-HPV-positive women[14].GYPC methylation showed a sensitivity of 83.3% for CIN2+ and 90.2% for CIN3+, with higher specificity than cytology for both endpoints[14].The combination of HPV16/18 and GYPC methylation was also evaluated and produced a CIN3+ sensitivity of

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95.1% and a negative predictive value of 99.4%.[14].Molecular triage has also been evaluated using HPV-positive samples in a cervical screening setting[15]. In a study of 1915 HPV-positive screening samples, genotyping showed slightly higher sensitivity than cytology but lower specificity, whereas methylation demonstrated higher specificity but substantially lower sensitivity [15]. Combining molecular methods increased positive predictive value but significantly reduced sensitivity[15]. The authors concluded that the evaluated molecular triage methods did not support replacement of cytology-based triage in the investigated screening setting [15].Taken together, the studies selected for this case demonstrate that NILM cytology and HR-HPV positivity can be further characterised using HPV genotype information, extended HPV genotyping, and molecular triage approaches [6,15].In particular, HPV39, HPV56, HPV66, and HPV68 are included within the extended genotype groups evaluated in studies of HPV-positive screening and Xpert HPV testing[7,10]. The present case therefore corresponds to an HR-HPV-positive/NILM screening pattern with detection in an extended genotype group, while the available studies evaluate genotype-specific risk and additional triage approaches for HPV-positive women [6,15].

Conclusion

This case demonstrates a discordant cervical screening result characterised by NILM cytology and positive high-risk HPV testing. Cytological examination identified no intraepithelial lesion or malignancy, while the Xpert® HPV HR assay detected HPV DNA within the HPV39/68/56/66 genotype group, with HPV16, HPV18/45, HPV31/35/33/52/58, and HPV51/59 not detected. Because the assay reports genotype groups rather than individual genotypes within the positive channel, the specific HPV genotype responsible for the positive result could not be determined from this test alone. The findings demonstrate that HPV positivity and cytological assessment provide different information within cervical cancer screening. The reviewed studies indicate that extended HPV genotyping and additional triage approaches, including cytology, p16/Ki67 dual staining, and molecular biomarkers, can further characterise risk among HPV-positive women. In the present case, the HR-HPV-positive/NILM pattern supports risk-based clinical follow-up according to applicable cervical cancer screening guidelines rather than a diagnosis of precancerous disease.

Conflict of Interest

The authors declare no conflict of interest.

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