

# Viral and cellular biomarkers used for improving prevention and management of cervical cancer

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## ABSTRACT

*Cervical cancer remains one of the preventable cause of women’s death, the second most common cause of death in Europe. Several screening tests for precancerous lesions are available, including cervicovaginal cytology and screening of high-risk human papillomavirus (HPV) strains – 16, 18, 31, 33. Cervical cytology (Babeș-Papanicolau smear test) remains the elected method for cervical cancer screening in many countries. Last decades, HPV DNA sequence was identified, including gene number and encoded proteins. HPV DNA gene expression involves the use of multiple promoters and complex patterns of splicing, which served as a model for the elaboration of cellular and molecular biomarkers for the identification. Herein, we present the usefulness and efficiency of viral and cellular biomarkers in improving the diagnosis and managing precursor lesions of cervical cancer.*

*Viral and cellular biomarkers can help differentiating low-grade lesion leading to a better diagnosis. HPV DNA tests, although a primary screening method in actual guidelines, can serve as a better screening test method. Other viral biomarkers that include E6 and E7 mRNA testing and determining the methylation of viral genes have good test screening results. Cellular biomarkers such as the immunocytochemical staining of p16INK4a, alone or combined with the proliferation marker Ki-67, can reveal anomalies in the cell cycle. The E4 protein, strongly expressed in productive infections, can prove a transforming infection when it is absent.*

*In recent years, detection of the TERC gene and oncogenetic microRNAs expressed early in carcinogenesis before clinical findings show great promise in differentiating lesions likely to progress to cancer.*

*In conclusion, biomarkers are more powerful instruments to enhance early pre-cervical cancer lesions diagnosis, which allow better prognosis and more reliable treatment.*

**Keywords:** cervical cancer screening, E6 mRNA, E7 mRNA, p16INK4a, Ki-67

## INTRODUCTION

Cervical cancer remains the second most common cause of women’s death in Europe (1). Romania exhibits a mortality four times higher than Western Europe

(2). The persistence of Human Papilloma Virus (HPV) infection is responsible for over 99% of cervical cancer cases (3). Nevertheless, most HPV-induced lesions will not progress to cancer (4). Genital HPV infection is the most frequent sexually transmitted infection globally,

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and most sexually active women will be infected with HPV at some periods in their lives (5). HPV is a group of more than 200 related viruses, and at least 40 types infect the genital female tract (6); about 14 types are considered oncogenic, frequently HPV 16, 18, 31, 33. HPV is a small, non-enveloped, epitheliotropic viruses from the *Papillomaviridae* family with approximately 8 Kbp circular double-stranded DNA (7,8).

The genome encodes six early proteins (E1, E2, E4, E5, E6 and E7) that are responsible for virus replication and two late proteins (L1 and L2), which are the major and minor viral capsid proteins, respectively (9-11).

Carcinogenesis is a complex process from the precancerous lesion (high-grade squamous intraepithelial lesion (H-SIL), low-grade squamous intraepithelial lesion (L-SIL) to cancer. Last decades, HPV DNA sequence was identified, including gene number and encoded proteins. HPV DNA gene expression involves the use of multiple promoters and complex patterns of splicing, which served as a model for the elaboration of cellular and molecular biomarkers for the identification

Screening for precancerous lesions provides an excellent window for clinical prevention, diagnosis, and optimal treatment. Last decades, the natural history of cervical cancer, genomic and proteomic human papillomavirus (HPV) identification and vaccination may contribute largely to understand this pathology.

LAST Project established new classification criteria for cervical squamous cancer, which is classified into low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL). LSIL represents non-carcinogenic human papillomavirus (HPV), resolved without treatment. LSIL is the same as cervical intraepithelial neoplasia (CIN) I in the traditional CIN classification standard. HSIL (same as CIN II and III) is a precancerous lesion and often requires surgical intervention to inhibit further progression to cervical squamous cell cancer. Comparing triage algorithms using HPV DNA genotyping, HPV E7 mRNA detection and cytology in high-risk HPV DNA-positive women was reported (12).

If high-grade squamous intraepithelial lesion (HSIL) and Atypical Squamous Cells, Cannot Rule Out High Grade Squamous Intra-epithelial Lesion (ASC-H) lesions will be immediately sent for colposcopy, atypical squamous cell indetermined signification (ASC-US) and low-grade squamous intraepithelial lesion (LSIL) results require appropriate triage methods (13). Even though new screening and diagnostic technologies, viral and cellular biomarkers, are rapidly emerging, cytology remains the preferred method in many states. Despite its low sensitivity (53%), repeat cytology at low intervals ensures its efficacy (14).

Therefore, more objective (viral, molecular) triage markers are suitable for automated and standardized screening processing.

Herein, we present the usefulness and efficiency of viral and cellular biomarkers in improving the diagnosis and the management of precursor lesions of cervical cancer.

## VIRAL BIOMARKERS

### HPV DNA testing

This is the most used triage method worldwide (15). HPV detection increases with disease severity (16). Low-grade disease are typically characterized by high-risk HPV types, including HPV 16 (3.2%), 18 (1.4%), 31 (0.8%), and 58 (0.7%). HPV high-risk types are founded in 50-70% of CIN1/LSIL (i.e. low-grade neoplasia). In CIN2, there is 85% positivity for HPV and in CIN3 and invasive cervical cancer, the HPV positivity rises to between 90% and 100% (17).

HPV DNA test advantage includes high sensitivity and similar specificity (96% versus 53%, 90% versus 96% respectively) and the objective and reproducible result (18). Two technologies have been approved for HPV DNA testing: genomic amplification through polymerase chain reaction (PCR) and signal amplification hybridization for HPV (digene hybrid capture 2 – hc2). HPV (Digene Hybrid Capture 2 – hc2 is the gold standard for new technologies, as they must fulfil criteria in comparison to hc2: similar sensitivity, specificity and inter- and intralaboratory reproducibility (19). New guidelines recommend HPV DNA testing as a primary screening at a five-year interval (20). Still, despite the high sensitivity of this test, it cannot differentiate between a transitory and a persistent HPV infection, and it is imperative to be combined with cytology (21).

The production of new viral particles characterizes a transitory, productive HPV infection, thus by the expression of the late genes L1 and L2, which are necessary for viral assembly. Late region methylation, with the consecutive inactivation of the L1 and L2 genes, reveals the decrease of viral copies production and potential evolution to precursor and invasive lesions; it is a potential triage biomarker (22).

Methylation tests can also be applied for the host's genes (determination of FAM19A4 and has-mir-124-2 hypermethylation) (23) or simultaneously for the viral and host DNA (the S5 panel for methylation of late regions of HPV 16, 18, 31, 33 and the host gene EPB41L3) (24).

### E6 and E7 messenger RNA testing

Another potential marker of a transforming infection is E6 and E7 messenger RNA testing. Hyperexpression of these two oncoproteins in persistent infections leads to deregulation of the cell cycle and progression to invasive lesions (20). Studies have shown that mRNA

testing is appropriate as a triage method for precursor lesions (20) and a primary screening (25). Many reports have shown that the level of hrHPV E6/E7 transcripts correlates with severity of histological abnormality (26-28).

## CELLULAR BIOMARKERS

The p16<sup>INK4a</sup> protein is a cell cycle inhibitory molecule – a stress stimulus induces it to block the inactivation of the tumor suppressor protein pRb (Retinoblastoma protein). The E7 oncoprotein induces such a stress stimulus and, at the same time, inactivates pRb. The result is the accumulation of p16<sup>INK4a</sup> as well as the continuation of cell division (29).

p16<sup>INK4a</sup> can be used in triaging ASC-US and LSIL cytology, alone and combined with Ki-67, a proliferation marker that is present in all phases of the cell cycle with the exception of G<sub>0</sub> (30). A meta-analysis of Peeters et al. (2019) shows that, when compared to DNA testing, p16 and p16/Ki-67 double staining have a lower sensitivity, but higher specificity in triaging ASC-US and LSIL lesions, having the potential of limiting the number of unnecessary interventions (31).

p16 /Ki-67 double staining test in Romania is known as commercial CINTEC PLUS or CIN2+ tests.

Determining the E4 biomarker simultaneously with MCM (minichromosome maintenance protein) relies on a similar principle. The E4 protein is abundantly expressed in productive infections, but MCM is a surrogate marker for E6/E7 expression. These two molecules are seldom found in large quantities in the same cell at the same time, which serves a promising principle for a new triage test (32).

## OTHER TECHNOLOGIES

Detection of the TERC gene from cytology specimens, which is early expressed in carcinogenesis along with the amplification of the 3q chromosomal arm, has proved high sensitivity and specificity in differentiating high-grade from low-grade lesions, and it could be useful in triaging ASC-US and LSIL lesions (33).

MicroRNAs (miRNAs) are small RNA molecules that have a role in modulating gene expression (20). E6 and E7 oncogenes of HPV16 and 18 amplify the expression of oncogenic miRNAs, which can be found in an increasing proportion as the lesion severity increases (34).

Techniques that allow self-sampling for HPV testing are a potential solution for the low participation in screening programs; their sensitivity and specificity for CIN2+ diagnosis have similar values to the samples taken by a clinician: 93,1% versus 96,1%, 94,0% versus 94,3%, respectively (35).

## CONCLUSIONS

Biomarkers are helpful for the improvement of cervical cancer screening and management. However, there are not routinely introduced due to advanced technologies and high costs. HPV DNA testing is increasingly used as a primary screening method in cervical cancer and precursor lesions. Still, repeated Babes-Papanicolau cytology, a cost-effective triage strategy, remains the elected method in many countries as biomarkers serve to complete it in better diagnosing equivocal and abnormal results.

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