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Translational Insights into Cervical Cancer Screening: The Role of p16INK4a and Ki-67 in Early Detection

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Abstract

Despite the global coverage of the early detection programs, cervical cancer is still one of the most common causes of death among women worldwide. The integration of Pap test in the healthcare systems worldwide has led to major advances in the diagnosis of premalignant changes in the cervix, although there are limitations regarding the sensitivity of the test. Due to the somewhat lower sensitivity and specificity of the Pap test, the Human Papillomavirus (HPV) (test has been adopted as the first-tier screening method. The further evaluation of the findings is followed by the various complementary techniques and methods to diagnose patients or quantify the risk of developing high-grade cervical intraepithelial lesions. These techniques are increasingly being investigated to provide specific and reliable final diagnosis and instruct the further treatment. This review summarizes the biological basis of p16 and Ki-67 expression, their correlation, and their diagnostic role in the triage of HPV-positive women. The analysis includes results from major clinical trials and meta-analyses, which demonstrate that dual immunostaining of p16/Ki-67 provides higher sensitivity for detecting CIN2+/CIN3+ compared to cytology alone, with an acceptable trade-off in specificity. In conclusion, dual staining represents a reliable complementary tool for the evaluation of abnormal cytological findings, improving early detection of cervical cancer and guiding the appropriate management and treatment of patients.

Introduction

Cervical cancer continues to pose a major public health issue, both globally and locally, despite the availability of prevention strategies and early detection tools. In an effort to reduce its incidence, screening programs are continually being evaluated and improved from medical, technical, and socioeconomic perspectives.

One of the earliest and most widely used screening methods is cervical smear cytology. It has been utilized for decades in many countries with organized screening programs. The Bethesda system (Nayar and Wilbur, 2015) is the current standard for classifying cytological findings. Revisions made in 2001 and 2014 have introduced important changes aimed at aligning cytological classifications more closely with biologically significant cervical abnormalities. The introduction of liquid-based cytology (LBC) has enhanced sample quality, lowered the number of inadequate samples, and led to an increased detection of premalignant changes in laboratories utilizing this method.

Routine screening methods for cervical cancer include the conventional Pap test, LBC, and HPV deoxyribonucleic acid (DNA) testing. The Pap test shows a wide range in sensitivity, from 20% to 70%, largely influenced by sample quality and cytologist expertise (Luria et al., 2023). Despite this variability, the Pap test has played a crucial role in decreasing cervical cancer mortality, especially when combined with HPV testing (Kumar et al., 2024).

Numerous testing approaches have been described (zur Hausen, 1991; Kjaer, 2002), but a substantial number of cervical cancer cases still go undetected in time. This underlines the ongoing need for more efficient diagnostic tools to detect premalignant lesions with a high risk of progression. These precancerous cervical changes, also known as premalignant lesions, occur as epithelial dysplasia, progressing through mild to severe stages and potentially leading to carcinoma in situ. These changes are commonly linked to persistent infection with high-risk HPV types—specifically types 16, 18, 31, 33, among others. These types are detected in almost 99% of high-grade lesions and are therefore classified as oncogenic (zur Hausen, 1991). In most countries, combining HPV testing with cytology has become a standard approach for rapid and effective screening in high-risk populations (Arbyn et al., 2021).

Despite the current practice based on Pap test cytology, there is still a need for additional methods that could improve the accuracy and sensitivity of diagnosis. Biomarkers such as p16 and Ki-67, especially their dual coexpression, are of great importance for the identification of high-grade cervical lesions.

The introduction of these biomarkers into routine practice has the potential to improve several important aspects of prevention and treatment. First, it allows for more accurate risk stratification in HPV-positive women, reducing the number of unnecessary colposcopies and invasive procedures. Second, combining immunocytochemical methods with HPV testing and genotyping improves triage and enables earlier detection of lesions with the highest malignant potential. This reduces the risk of overlooking high-risk patients, which is often a limitation of cytology or HPV testing alone. Third, the integration of these methods contributes to the standardization and objectification of diagnostics, especially in conditions where the quality of cytology may vary.

Therefore, the aim of this review is to summarize the biological background of p16 and Ki-67

expression, evaluate their clinical relevance, and critically analyze the available evidence for the use of dual staining as a triage tool in HPV-positive women with abnormal cytology. By integrating data from large clinical trials and meta-analyses, this article attempts to highlight the diagnostic accuracy, advantages, and limitations of dual staining compared to conventional methods and to explain its potential role in routine cervical cancer screening and treatment decision-making.

Cervical cancer as a global women's health challenge

Cervical cancer is ranked as the fourth leading cause of cancer-related deaths among women worldwide, even with the availability of advanced diagnostic methods (Sung et al., 2021). The burden is especially pronounced in low- and middle-income countries (Arbyn et al., 2021). According to the World Health Organization (WHO) (WHO, 2020), over 85% of cervical cancer deaths occur in regions where screening is inconsistent, poorly funded, or completely absent.

Highly developed nations have established organized screening programs that often use HPV testing as the primary method, sometimes combined with cytology. In contrast, developing countries usually rely on opportunistic screening. For instance, Croatia has practiced opportunistic screening since the late 1960s, with an estimated 70% of the target female population undergoing Pap tests every three years. This practice has led to a notable decline in both incidence and mortality rates from cervical cancer (Pajtler et al., 2007).

Disparities between countries in different stages of development contribute significantly to global health inequalities. Poorly structured screening programs not only increase disease burden but also bring about considerable economic costs, as premature death and reproductive health issues reduce productivity (WHO, 2021). The lack of preventive services stems from multiple issues: limited healthcare infrastructure, insufficient public awareness and education, cultural and social barriers, scarce financial resources, and inadequate political support (Arbyn et al., 2021). Another complicating factor is labor migration, which disrupts continuity in care and increases exposure to sexually transmitted infections, including those that may go undetected due to inaccessible health services or limited public information.

Major risk factors for cervical cancer

HPV, a sexually transmitted virus, is a well-established cause of cervical cancer. If left untreated, persistent infection can progress to cancerous changes. Multiple risk factors can accelerate this process. Early onset of sexual activity increases exposure time to HPV and other Sexually Transmitted Disease (STD)s (Bosch et al., 2002). A high number of sexual partners raises infection risk (Bruni et al., 2019), while diseases that weaken the immune system, such as Human Immunodeficiency Virus (HIV) or immunosuppression, further elevate susceptibility (Clifford et al., 2017). Smoking also plays a role by inducing local immunosuppression and genotoxic stress in cervical tissue (Castellsagué et al., 2006). Long-standing inflammation from bacterial vaginosis or other STDs damages tissue and may act as a cofactor in cancer development (Amabebe et al., 2018; Pourmollaei et al., 2020). Irregular screening significantly increases the likelihood of undetected precancerous lesions progressing to invasive cancer (Arbyn et al., 2020), and women from lower socioeconomic

backgrounds often face barriers to accessing timely screening and treatment (Louie et al., 2009).

The effects of these pathogens on cervical tissue are both direct and indirect. Direct effects involve structural damage from enzymes and toxins, while indirect effects stem from the host immune response. Persistent immune activation releases cytokines, interleukins, and other inflammatory mediators, which can damage cervical epithelial cells (Pourmollaei et al., 2020; Amabebe et al., 2018). This underscores the importance of improving and streamlining diagnostic methods for pathogens involved in cervical carcinogenesis.

Pap test in the detection of cervical carcinoma

Cervical cancer is one of the most preventable cancers thanks to early detection methods like the Pap test, which has been in clinical use for over 70 years. The Pap test analyzes cells from the surface of the cervical epithelium to identify premalignant or malignant changes. There are two cytology approaches: conventional cytology, where samples are smeared directly onto slides, LBC, which involves suspending cells in a fluid medium. LBC offers several advantages, such as immediate fixation, cleaner backgrounds, and a reduction in inadequate samples, all of which improve diagnostic quality (Davey et al., 2006). LBC also allows for molecular and immunostaining tests without needing a second patient visit.

Comparative studies of LBC and conventional cytology have shown that while both methods are similarly effective at diagnosing high-grade squamous intraepithelial lesions (HSIL), LBC detects more mild abnormalities and provides better sample adequacy (Davey et al., 2006; Davey et al., 2007). The traditional cervical intraepithelial neoplasia (CIN) classification system has been replaced by the Bethesda system, which distinguishes between low-grade squamous intraepithelial lesions (LSIL) and HSIL and is now the global standard.

The use of dual biomarkers like p16 and Ki-67 represents a promising advancement. This immunocytochemical technique, which detects both proteins in cervical epithelial cells, offers higher sensitivity than traditional cytology, though with slightly reduced specificity. It supports earlier detection and more targeted treatment of cervical cancer, although careful interpretation remains essential (Ouh et al., 2024).

HPV testing as indispensable tool for risk stratification for cervical cancer

HPV is a key driver of oncogenesis in cervical cells, altering tumor suppressor genes and proliferative markers. All human papillomaviruses share a similar genome structure. Due to its small genome size, HPV contains only six to eight open reading frames (ORFs) on a single strand of viral DNA (McBride, 2017). The viral genome includes conserved genes for replication Early protein (E) (E1, E2) and capsid proteins Late protein (L) (L1, L2) (Burley et al., 2020), while E6 and E7 oncoproteins interact with host tumor suppressors (Sen et al., 2018).

HPV infects basal epithelial cells in the cervical transformation zone after microtrauma. It enters the basal layer via membrane receptors and can persist as episomes, with gene expression regulated by E1 and E2. After multiple cell divisions, the viral genome may integrate into the host genome (Cosper et al., 2021), often fragmenting in the E1 or E2 regions. Loss of E2 function leads to uncontrolled E6/E7 expression.

E6 degrades p53, disrupting apoptosis and reducing p53 levels, resulting in cell cycle deregulation. E7 inactivates the Rb tumor suppressor, promoting unchecked cell proliferation.

Unlike cytology, which has lower sensitivity (Walker et al., 2006; Almonte et al., 2016; Castle et al., 2011), HPV testing shows higher sensitivity but lower specificity in detecting premalignant lesions. Most HPV infections resolve spontaneously, particularly in women under 30, reducing the test's specificity (Maver et al., 2020). However, a negative HPV test offers a more reliable indication of the absence of premalignant lesions (Ronco et al., 2014).

Many countries now prefer HPV testing as the primary screening method. To reduce unnecessary colposcopies, new strategies include HPV genotyping, methylation testing, and p16/Ki67 dual immunostaining (Dovnik et al., 2023). Although histology remains the gold standard for diagnosing epithelial changes, it is subject to interobserver variability (Chartian et al., 2025), emphasizing the need for new, more objective diagnostic tools (Sarma et al., 2021).

Cervical cytology findings significantly influence treatment and diagnostic steps. Clear abnormalities require complementary diagnostic methods to guide therapy (Anand et al., 2021). Alongside conventional screening, HPV testing for all sexually active women is now essential (WHO, 2021), as persistent high-risk HPV is a major cause of progression from premalignant to invasive disease. Genotyping for high-risk HPV types enhances risk assessment. Oncogenes E6 and E7 are central in this process by disrupting cell cycle regulation (Hu and Ma, 2018).

Among adjunctive techniques, dual-staining cytology with p16/Ki67 stands out for its high sensitivity and specificity in identifying high-grade lesions and assessing risk (Magkana et al., 2022). Borderline cytology findings require further triage, as morphology alone cannot predict lesion progression. Thus, p16/Ki67 staining provides an important diagnostic enhancement (Ikenberg et al., 2013). This method detects co-expression of p16 and Ki67 within the same cervical epithelial cell, aiding precise detection of high-risk lesions (Dovnik et al., 2023).

Immunocytochemical tests for p16 and Ki-67 are based on the principle of specific antigen-antibody reactions, using highly specific monoclonal antibodies directed against human proteins p16INK4a and Ki-67. The staining method can be applied to samples previously stained using the Papanicolaou method, to fresh samples, and to samples obtained using the LBC method. The most commonly used tests include the mouse monoclonal antibody clone E6H4, which recognizes the p16INK4a protein, and the recombinant rabbit antibody clone 274-11AC3V1, which binds to the proliferation marker Ki-67. After binding the antibody to the target antigen, visualization is achieved using enzyme-chromogenic systems, resulting in a brown stain in the cytoplasm and/or nucleus of p16-positive cells and a red signal in the nucleus of Ki-67-positive cells. The key interpretative value of this method lies in the detection of co-expression of p16INK4a and Ki-67 in the same cell, which is considered a reliable marker of HPV-induced transformation and the progressive potential of high-grade lesions (CIN2+) (Schmidt et al., 2011). In order to reduce the possibility of technical errors and ensure the accuracy of the results, appropriate positive and negative quality controls are performed in each staining cycle. An example of double staining is shown in Figure 1.

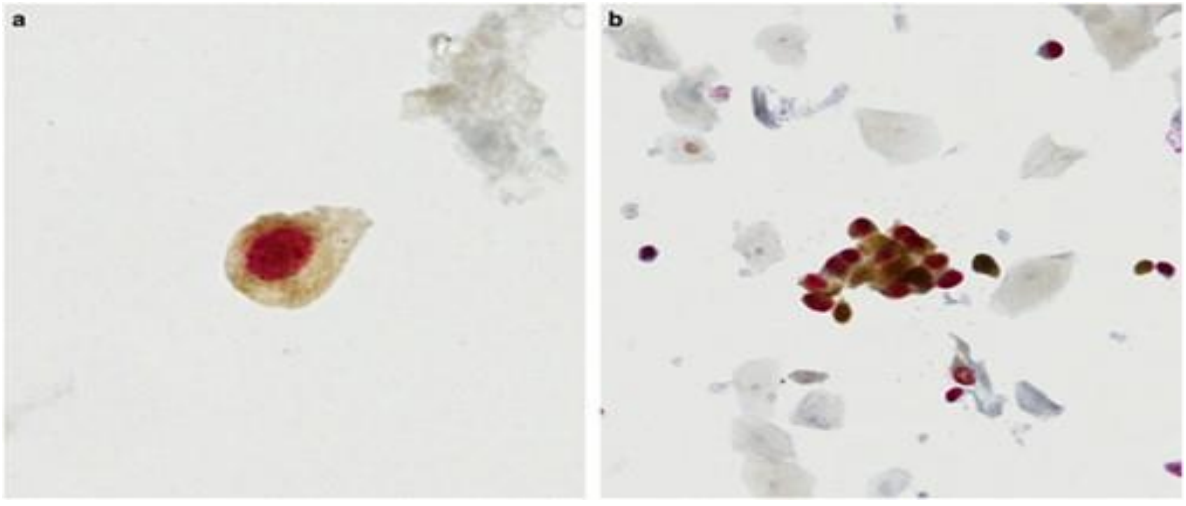

Figure 1. Example of double staining p16/ki67 in cervical cytology. a) p16/ki67 positive single cell on double staining. b) Cluster of cells positive on double staining. The case is considered positive if one or more cells are stained red with Ki-67 nuclear stain and brown with p16 cytoplasmic stain (Renee et al., 2017)

Biological role of p16/Ki67

The cell cycle is a complex process that controls the activity of numerous positive and negative protein regulators. Key molecular effectors that regulate specific phases of the cell cycle include cyclins, cyclin-dependent kinases (CDKs), and their inhibitors. Their main task is to ensure the correct duplication of DNA and its division into two new cells (Hall et al., 1996). During cell division, cells transition from a resting state (G0 phase) to the G1 phase, also known as the "critical point," because this is when proliferation begins. This is where mutations in regulatory proteins most often occur, which can result in the progression of the malignant process. The p16INK4a protein belongs to the CDK family. Its main function is to bind to CDK4/6, thereby preventing the formation of the CDK4/6–cyclin D complex. This process prevents the phosphorylation of the retinoblastoma (Rb) protein and blocks the transition of cells from the G1 to the S phase of the cell cycle.

In normal cells, p16INK4a is expressed at low levels and acts as a tumor suppressor. However, in cells infected with high-risk HPV types (especially 16 and 18), the viral oncoprotein E7 inactivates Rb, leading to compensatory overexpression of p16INK4a. This phenomenon makes p16INK4a a reliable biomarker for the detection of transformative lesions of the cervix. In many human carcinomas, p16 is often inactivated, and the loss of p16 function may be an early event in carcinogenesis (Liggett and Sidransky, 1998). This is also evidence of active expression of the viral oncoprotein E7 in dysplastic cells, or it can serve as a marker of HPV oncogenic activity, as it simultaneously confirms cell cycle dysregulation and transformative infection, which is strongly associated with premalignant changes (Clarke et al., 2021).

The Ki-67 protein is a nuclear protein associated with proliferation, expressed in all active phases of the cell cycle (G1, S, G2, and M), but absent in the G0 phase (Scholzen and Gerdes, 2000). In mitotic cells, Ki67 plays a key role in the formation of the pericromosomal layer (PCL) that surrounds condensed chromosomes. The localization of the Ki-67 protein is regulated during the cell cycle,

and its role and position are shown in Figure 2. This layer enables the proper distribution of mitotic chromosomes and prevents their aggregation (Cuylen et al., 2016). Because of this property, Ki-67 is widely used as a marker of proliferation in histopathological and cytological analyses.

In tumor cells, high levels of Ki-67 expression indicate increased proliferative activity and are associated with more aggressive biological behavior and poorer prognosis (Andrés-Sánchez et al., 2022).

Ki67 is also used to assess tumor malignancy because it is associated with differentiation, invasion, metastasis, and tumor prognosis (Ferrandina et al., 2001). Its expression depends on a precise balance between synthesis and degradation, and the half-life of Ki67 is only 1-1.5 hours (Halm et al., 2000).

Therefore, p16 and Ki67 proteins play a key role in cell cycle regulation, where detection of their overexpression can serve to better identify cells in the process of proliferation and malignant transformation (Alshenawy et al., 2014; Sangwaiya et al., 2018). This feature gives them a role as biomarkers for identifying premalignant and malignant changes, especially in the context of HPV infection. Precise regulation of these proteins is key to maintaining the balance between tumor suppression and aging, and their dysfunctional activity may be an early indicator of the development of malignant diseases.

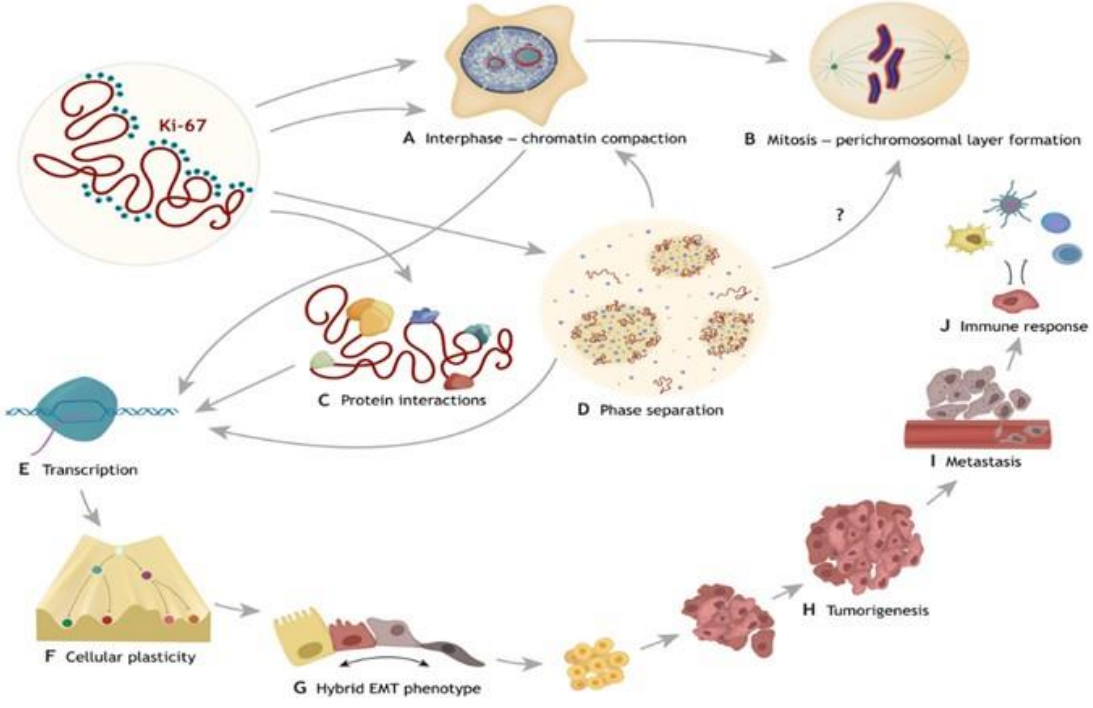

Figure 2. Localization and function of Ki-67 (Andrés -Sánchez, et al., 2022). During interphase (the resting phase of the cell), Ki-67 is located near the nucleolus (perinucleolar) and around the centromere chromatin (pericentromeric), where it participates in chromatin condensation. During mitosis (cell division), Ki-67 moves to the surface of the chromosome and enables the formation of the so-called pericromosomal envelope. Through numerous protein interactions and possible participation in phase separation of various subnuclear structures, Ki-67 organizes heterochromatin (densely packed genetic material) and regulates transcriptional (genetic) programs. This enables cellular plasticity—the ability of cells to adapt and change. In tumor cells, Ki-67 is essential for maintaining the so-called hybrid EMT phenotype (epithelial-mesenchymal transition), which is associated with invasiveness and cancer spread. As a result of all of the above, Ki-67 plays an important role in cell transformation, tumor development, metastasis, and the immune system's response to tumors (Andrés-Sánchez et al., 2022).

Application of p16/Ki-67 Double Staining in Cervical Cancer Screening Across Healthcare Settings

Persistent infection with high-risk HPV genotypes (H-HPV), particularly HPV 16 and 18, is the main cause of cervical carcinogenesis. In response, dual immunocytochemical staining for p16/Ki-67 has been incorporated into screening protocols. In 2019, a consensus was reached in the United States (US) on the management of HPV-positive patients with abnormal cytology, following which dual staining was approved for triage by the Food and Drug Administration (FDA) in 2020 (Perkins et al., 2020; Clarke et al., 2021). Portugal was one of the first European countries to include this method in its national guidelines and has shown that dual staining simplifies referral for colposcopy and safely extends follow-up intervals in HPV-positive women, reducing the need for annual testing (Sepodes et al., 2024).

The strongest evidence for the diagnostic value of dual staining with p16/Ki-67 comes from two large multicenter studies—PALMS and ATHENA.

- The PALMS study (Ikenberg et al., 2013) included 27,349 women in five European countries and showed that dual staining is significantly more sensitive than cytology (86.7% vs. 68.5%) in detecting CIN2+, while maintaining high specificity (95.2% vs. 95.4%). Compared to HPV testing, dual testing had lower sensitivity (84.7% vs. 93.3%) but higher specificity (96.2% vs. 93.0%), making it particularly useful for screening younger women, for whom HPV testing has limitations.
- The ATHENA study (Wright et al., 2017) included 7,727 HPV-positive women ≥ 25 years of age and retrospectively applied p16/Ki-67 double staining to LBC samples. The results showed higher sensitivity than cytology in screening for CIN3+ lesions (74.9% vs. 51.9%) with similar specificity (74.1% vs. 75.0%). When combined with HPV16/18 genotyping, the overall sensitivity for CIN3+ reached 86.8%, confirming the potential of this strategy to optimize referral for colposcopy.

These studies carry the most weight because they are multicenter, involve a large number of subjects, and use histologically confirmed results, making them the gold standard for evaluating the performance of dual staining.

In addition, numerous smaller studies and meta-analyses have further confirmed the usefulness of the method, albeit with limitations in terms of sample size and specificity of local populations:

- Secosan et al. (2022) showed in a cohort of women <30 years of age with ASC-US/LSIL that the combination of p16/Ki-67 and colposcopy provides the highest accuracy, while the addition of HPV genotyping improves specificity.
- Luttmer et al. (2016) emphasized the importance of combined strategies in triaging HPV-positive women.
- Renée et al. (2017; 2020) found that dual testing significantly increased sensitivity compared to cytology (92% vs. 93% for Pap test) with better specificity (61% vs. 49%). In combination with HPV16/18 genotyping, sensitivity increased to 97%.
- Voidăzan et al. (2022) extended the analysis to additional biomarkers (hTERC, fibronectin) and highlighted their potential in assessing individual risk and early detection of lesions in HPV-positive women.

- The meta-analysis by Bedon et al. (2024), which included 42 studies, showed an average sensitivity and specificity of double staining for CIN2+ of 87.7% and 76.7% and for CIN3+ of 89.7% and 79.6%. Combination with an HPV test further increased predictive accuracy, while the strong correlation of the biomarker with lesion severity further confirmed the clinical value of the test.

Overall, the results show that p16/Ki-67 double staining demonstrates robust performance in large multicenter studies that can be transferred to clinical practice, while smaller studies and meta-analyses confirm its consistent applicability, albeit with certain methodological limitations.

Other relevant biomarkers in subclassification of cervical intraepithelial lesions

To further improve diagnosis and triage of CIN2+ lesions, increasing attention is being paid to molecular biomarkers that complement cytology and HPV testing. Among the most promising are amplification of the human telomerase RNA component (hTERC) and fibronectin (FN1) expression.

Telomerase activity, which helps cells avoid senescence and apoptosis, is commonly upregulated in early cancer. hTERC, a key telomerase component, is closely associated with high-risk HPV-driven oncogenesis, particularly via E6/E7 oncoproteins. Amplification of hTERC is frequent in high-grade lesions and invasive cancer but rare in benign conditions, supporting its diagnostic and prognostic value. hTERC is also being studied as a triage tool for HPV-positive women with unclear cytology (Chen et al., 2012). FN1 an extracellular matrix glycoprotein, influences cell adhesion, proliferation, migration, and activates pathways such as focal adhesion kinase (FAK). FN1 is overexpressed in multiple malignancies, including cervical cancer (Chen et al., 2021). Preclinical data indicate FN1 enhances cervical cell viability and invasiveness through FAK signaling, while its downregulation promotes apoptosis (Chen et al., 2021). Serum studies in women with abnormal Pap tests support FN1's utility in early detection (Voidăzan et al., 2023). Though still under investigation, hTERC and FN1 hold promise for refining risk stratification and supporting personalized care in HPV-positive patients.

Discussion

Cervical cancer remains a largely preventable malignancy through organized, population-based screening and timely management of precancerous lesions. According to the 2021 WHO guidelines, the main aim of screening is to identify women infected with HR-HPV (genotypes and detect cervical intraepithelial neoplasia grade 2 or higher (CIN2+) early enough for treatment to prevent progression to invasive cancer.

Screening typically begins at age 25, regardless of sexual activity, and continues until age 65. Screening intervals and test types vary by age, past results, and national protocols. For women aged 25–29, cytology every three years is standard; for women aged ≥30, primary HR-HPV testing every five years is preferred, with co-testing (HPV + cytology) as an alternative. Where HPV testing isn't available, cytology remains acceptable.

Biomarkers now play an increasing role in triage, particularly dual immunocytochemical staining for p16^INK4a^ and Ki-67. Co-expression of these proteins in the same cell signals HPV-driven cell

cycle disruption and proliferation. Dual staining improves specificity for CIN2+ in HPV-positive women with borderline or mildly abnormal cytology (ASC-US or LSIL) and reduces unnecessary colposcopy referrals without compromising sensitivity (Wentzensen et al., 2012; Wright et al., 2017). Its clinical utility is well-documented, enhancing triage accuracy and efficient use of resources (Clarke et al., 2019).

Effective screening requires organization by health authorities, with central registries and proactive outreach. All women aged 25–65 should be invited via letters, digital alerts, or community services. Underserved populations—rural, low-income, or with limited access—require targeted efforts, including self-sampling and mobile screening units (Arbyn et al., 2014). All screening data (cytology, HPV, colposcopy, histology) should be centralized for follow-up and continuity of care.

Digital tools now assist in data processing, reminders, tracking follow-ups, and provider communication. Integrating public and private sectors into unified screening frameworks demands clear legal standards, protocols, data-sharing policies, and mutual accountability. Private sector involvement in quality assurance and financing may boost coverage and reduce disparities.

Despite benefits, p16/Ki-67 staining has limitations. Staining may be suboptimal in self-collected samples due to poor cellularity. Although specific for HPV-mediated transformation, p16 and Ki-67 can be overexpressed in non-neoplastic settings (e.g., metaplasia or inflammation), leading to false positives (Wentzensen et al., 2012). Interpretation is operator-dependent, with variability and a lack of universal scoring. More training and standardization are needed, especially for borderline cases (Renée et al., 2017).

Cost remains a major barrier, especially in low- and middle-income countries (LMICs), where infrastructure and trained personnel may be lacking. Although more expensive than cytology or HPV testing, p16/Ki-67 is rapid and highly sensitive for detecting CIN2+, potentially easing the psychological and financial burden of screening (Magkana et al., 2022). Still, it cannot replace biopsy and histopathological confirmation in more severe cases.

Application in glandular lesions, such as adenocarcinoma in situ (AIS), is promising but under evaluation. Glandular abnormalities are harder to detect cytologically. While p16/Ki-67 may help identify atypical glandular cells, it cannot rule out non-cervical glandular pathology in older women (Ryu et al., 2022). Still, most AIS or adenocarcinoma cases test positive, while low-grade or benign cases are typically negative (Jeromel et al., 2024), suggesting usefulness in detecting glandular disease pending further validation.

Voidăzan et al. highlighted the potential of combining p16/Ki-67 with hTERC and fibronectin to improve sensitivity and specificity, enhance triage accuracy, and reduce subjectivity. However, broader adoption of such panels awaits clinical validation and standardization.

Studies by Li Yu et al. (2019) and Wentzensen et al. (2012) support using p16/Ki-67 in high-risk groups. In women over 30, specificity increases, aiding stratification. In HPV-positive women with ASC-US or LSIL, dual staining reduces unnecessary colposcopies. Abbas et al. (2022) also found that HSIL may be missed by HPV testing alone, underscoring the role of immunocytochemistry.

In conclusion, p16/Ki-67 dual staining is a robust, evidence-based method that enhances HPV-based

cervical screening and triage. While it improves diagnostic accuracy and reduces overtreatment, challenges such as cost, accessibility, and standardization must be addressed to enable broader integration. Ongoing research, policy development, and innovation remain essential for embedding this biomarker into scalable and equitable prevention strategies.

Conclusion

Dual immunostaining for p16 and Ki-67 enhances cervical cancer screening by improving detection of high-grade lesions (CIN2+, CIN3+, AIS), which precede both squamous and glandular carcinomas. Co-expression of p16 (a surrogate for HPV-mediated cell cycle deregulation) and Ki-67 (a proliferation marker) signifies HPV-driven oncogenic transformation. While the assay demonstrates high sensitivity and negative predictive value (NPV), specificity remains suboptimal, necessitating cautious interpretation. Integration of p16/Ki-67 staining with HPV testing and cytology augments diagnostic precision and reduces unnecessary colposcopies. However, clinical implementation requires further validation regarding cost-effectiveness, standardization, and triage algorithms. Additional biomarkers such as hTERC and fibronectin may further refine risk stratification and support a personalized approach to screening. Importantly, p16/Ki-67 positivity obviates the need for morphologic interpretation, offering workflow advantages. Despite its demonstrated efficacy in detecting dysplasia and early-stage carcinoma, p16/Ki-67 dual staining should serve as an adjunct rather than a replacement for existing screening modalities. Critical knowledge gaps persist regarding appropriate risk stratification for HPV-positive/p16-Ki-67-negative women, as well as the evidence-based determination of optimal intervals for repeat testing. Evidence suggests dual staining could halve colposcopy referrals, particularly in women ≥30 years, though benefits must be balanced against assay-related costs. Prospective studies are warranted to establish long-term predictive value and refine molecular triage protocols.

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Authors' contributions

Irma Mukic: Research concept and design, manuscript writing.

Dženita Kurtćehajić: Manuscript revision.

Ines Krivak Bolanča: Supervision of the work, approval of the final version of the manuscript.

Lejla Pojskić: Supervision of the work, approval of the final version of the manuscript.

Conflict of interest

Authors declare no conflict of interest in relation to the publication of this article.

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