Clinical Efficacy and Innovative Applications of Cervical Cancer Screening Technologies: Multi-dimensional Analysis and Prospects Postprint

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Abstract

Cervical cancer is the second most common cancer among women globally, with high incidence and mortality rates particularly in low- and middle-income countries, posing a serious threat to women' s lives. Early screening and diagnosis are crucial for reducing the incidence and mortality of cervical cancer. This paper comprehensively reviews the current application status of various technical means in the field of cervical precancerous screening, covering Pap smears, liquid-based cytology (LBC), P16/Ki-67 dual staining, HPV E6/E7 mRNA testing, PAX1 gene methylation testing, HPV integration testing, self-sampling, and artificial intelligence-assisted screening. It provides an in-depth analysis of the clinical efficacy and limitations of each screening technology and looks forward to future technological innovations and research directions that are expected to achieve breakthroughs. The aim is to provide a scientific basis for clinical practice, assist in the precise selection of appropriate screening strategies, and achieve early detection and personalized intervention for cervical cancer, thereby effectively reducing its incidence and mortality.

Full Text

Preamble

Clinical Efficacy and Innovative Applications of Cervical Cancer Screening Technologies: A Multidimensional Analysis and Outlook

Abstract

Cervical cancer remains a significant global public health challenge. Early screening and intervention are critical strategies for reducing the incidence and

mortality of this disease. This review systematically analyzes the clinical efficacy of traditional screening methods, such as the Papanicolaou (Pap) smear, liquid-based cytology (LBC), and human papillomavirus (HPV) testing. Furthermore, it explores the innovative applications of emerging technologies, including HPV E6/E7 mRNA testing, DNA methylation markers, and artificial intelligence (AI)-assisted pathological diagnosis. By evaluating the strengths and limitations of various screening modalities across different clinical contexts, this paper provides a multidimensional perspective on the current landscape and future directions of cervical cancer screening, aiming to inform the development of more precise and efficient screening programs.

1. Introduction

Cervical cancer is one of the most common malignancies affecting the female reproductive system worldwide. According to global cancer statistics, its incidence and mortality rates remain high, particularly in developing countries where access to organized screening programs is limited. The natural history of cervical cancer involves a long-term progression from persistent high-risk HPV infection to cervical intraepithelial neoplasia (CIN) and, eventually, invasive carcinoma. This extended preclinical phase provides a vital window for effective screening and early intervention.

Over the past few decades, screening technologies have evolved from morphological assessments to molecular-level detection. While traditional cytology has significantly reduced the burden of cervical cancer in many regions, the integration of molecular biology and computational science is driving a paradigm shift toward more personalized and accurate screening strategies.

2. Clinical Efficacy of Traditional Screening Technologies

2.1 Cytology-Based Screening The introduction of the Pap smear in the mid-20th century marked the beginning of modern cervical cancer screening. Although it has been instrumental in reducing mortality, traditional Pap smears are limited by relatively low sensitivity and high inter-observer variability. Liquid-based cytology (LBC), such as ThinPrep (TCT) and SurePath, was developed to address these issues by improving sample collection and reducing obscuring factors like blood and mucus. While LBC enhances the quality of the slides, meta-analyses suggest that its sensitivity for detecting CIN2+ is comparable to or only slightly higher than that of the conventional Pap smear.

2.2 HPV DNA Testing The discovery that persistent infection with high-risk HPV (hr-HPV)

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Abstract

Cervical cancer is the second most common cancer among women globally, characterized by high incidence and mortality rates particularly in low- and middle-income countries, posing a severe threat to women' s lives and health. Early screening and diagnosis are critical for reducing the morbidity and mortality associated with this disease. This paper provides a comprehensive review of the current application status of various technical modalities in the field of cervical precancerous screening. These include Pap smears, liquid-based cytology (LBC), P16/Ki-67 dual staining, HPV E6/E7 mRNA detection, PAX1 gene methylation testing, HPV integration detection, self-sampling, and artificial intelligence-assisted screening. We conduct an in-depth analysis of the clinical efficacy and limitations of each screening technology and look forward to future technological innovations and research directions. This review aims to provide a scientific basis for clinical practice, assist in the precise selection of appropriate screening strategies, and facilitate the early detection and personalized intervention of cervical cancer, thereby effectively reducing its incidence and mortality.

Keywords: Cervical cancer; Screening; P16/Ki67 dual staining; HPV E6/E7 mRNA detection; PAX1 gene methylation; HPV integration; Self-sampling

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Introduction

Cervical cancer remains a significant global health challenge. Despite advancements in medical science, the burden of this disease remains disproportionately high in developing regions. The transition from human papillomavirus (HPV) infection to invasive cervical cancer is a slow process, typically spanning several years to decades, which provides a critical window for early intervention. Effective screening programs have been proven to significantly reduce the incidence of invasive disease by identifying precancerous lesions such as cervical intraepithelial neoplasia (CIN).

Current Screening Modalities and Clinical Efficacy

Cytology-Based Screening: Pap Smear and LBC

Traditional Pap smears have historically been the backbone of cervical cancer screening. While cost-effective, they are limited by relatively low sensitivity and high inter-observer variability. Liquid-based cytology (LBC) was developed to improve specimen quality by reducing obscuring factors like blood and mucus. Although LBC enhances the detection rate of glandular cell abnormalities, its sensitivity for high-grade lesions remains comparable to high-quality conventional smears.

Molecular Biomarkers: P16/Ki-67 Dual Staining

The co-expression of P16 and Ki-67 serves as a robust biomarker for cell cycle dysregulation caused by high-risk HPV (hr-HPV) transforming infections. P16 is a tumor suppressor protein that overexpresses in response to E7-mediated pRb degradation, while Ki-67 is a marker of cellular proliferation. Their simultaneous presence within a single cell indicates a loss of cell cycle control, providing higher specificity than HPV DNA testing alone in identifying high-grade CIN (CIN2+).

HPV E6/E7 mRNA Detection

While HPV DNA testing identifies the presence of the virus, it cannot distinguish between transient and persistent transforming infections. HPV E6/E7 mRNA detection targets the oncogenic transcripts responsible for malignant transformation. This method offers higher positive predictive value (PPV) for high-grade lesions compared to DNA testing, potentially reducing the rate of unnecessary colposcopies and over-referral.

Epigenetics: PAX1 Gene Methylation

DNA methylation is an early event in cervical carcinogenesis. The methylation status of the PAX1 gene has emerged as a promising biomarker. Research indicates that PAX1 methylation levels correlate significantly with the severity of cervical lesions. As an objective molecular marker, it minimizes the subjectivity inherent in cytological interpretation and can serve as an effective triage tool for hr-HPV positive women.

HPV Integration Detection

The integration of the HPV genome into the host cell chromosome is a critical step in the progression from CIN to invasive cancer. Detecting the physical state of the HPV genome (episomal vs. integrated) provides valuable prognostic information. Advanced sequencing technologies now allow for the precise mapping of integration sites, which may help identify patients at higher risk of rapid disease progression.

Innovative Applications and Future Directions

Self-Sampling and Accessibility

To increase screening coverage, particularly in under-resourced areas or among underscreened populations, self-sampling for HPV testing has gained traction. Clinical studies demonstrate that HPV testing on self-collected vaginal samples is nearly as sensitive as clinician-collected cervical samples. This approach addresses barriers such as cultural sensitivities, lack of transport, and healthcare provider shortages.

Artificial Intelligence in Cervical Screening

The integration of artificial intelligence (AI) and deep learning algorithms into digital pathology and colposcopy is transforming the screening landscape. AI-assisted systems can analyze LBC slides with high precision, reducing the workload of pathologists and minimizing human error. Furthermore, AI-enhanced colposcopy provides real-time diagnostic support, improving

Abstract

Cervical cancer is the second most common malignancy among women worldwide, with particularly high incidence and mortality rates in low- and middle-income countries, posing a significant threat to women s health. Early screening and diagnosis are critical for reducing the incidence and mortality of cervical cancer. This article comprehensively reviews the current applications of various screening modalities in the field of cervical precancerous lesion detection, including Papanicolaou (Pap) smear, liquid-based cytology (LBC) , p16/Ki-67 dual staining, HPV E6/E7 mRNA testing, PAX1 gene methylation analysis, HPV integration detection, self-sampling, and artificial intelligence (AI) -assisted screening. The clinical performance and limitations of each screening technique are critically analyzed. Furthermore, potential technological advancements and future research directions are discussed to provide a scientific foundation for clinical practice, facilitate the selection of optimal screening strategies, and enable early detection and personalized intervention, thereby effectively reducing the incidence and mortality of cervical cancer.

Key words Cervical cancer; Screening; P16/Ki67 double staining; HPV E6/E7 mRNA testing; PAX1 gene methylation; HPV integration; Self-sampling; Artificial intelligence WEN S Y, YANG J, DUAN S Q, et al. Clinical efficacy and innovative applications of cervical cancer screening technologies: a multidimensional analysis and outlook[J]. Chinese General Practice, 2025. [Epub ahead of print] Editorial Office of Chinese General Practice. This is an open access article under the CC BY-NC-ND 4.0 license.

Malignant tumors pose a severe threat to human life and safety, having become a major global public health challenge. With changes in lifestyle and economic development, both the incidence and mortality rates of malignant tumors are on an upward trend. Cervical cancer is the second most common cancer among women worldwide; in low- and middle-income countries, it accounts for 90% of female cancer deaths. In 2020, there were approximately 604,000 new cases of cervical cancer and 342,000 deaths globally. In 2023, the United States reported 14,000 new cases and 4,310 deaths. According to the latest data from the National Cancer Center of China, cervical cancer ranked 5th in incidence and 6th in mortality among female malignant tumors in 2022, with both rates showing an increasing trend. While the prognosis for early-stage cervical cancer and precancerous lesions is favorable, late-stage disease presents significant treatment challenges and poor outcomes. Therefore, cancer prevention, early screening,

and diagnosis are of paramount importance.

Cervical cancer primarily originates at the squamocolumnar junction of the cervix, and its primary etiology is persistent infection with high-risk human papillomavirus (HPV). During the initial stages of viral infection, abnormal proliferation occurs in the cervical region, known as cervical precancerous lesions. Although most infections resolve naturally within a few years, persistent infection can lead to high-grade cervical precancerous lesions, such as cervical intraepithelial neoplasia grade 2 (CIN 2) and grade 3 (CIN 3). Approximately 30% of CIN 3 lesions progress to invasive cancer within 30 years. This slow disease progression provides numerous opportunities for these lesions to be detected and treated, thereby intercepting the development of invasive cancer.

While the prevalence and detection rates of high-risk HPV infection are relatively high in female cervical cancer screening, the probability of these infections progressing to malignancy is low. Despite the high sensitivity of HPV testing, infections with more than a dozen types of oncogenic HPV are common and often benign. Consequently, to achieve more precise screening and predictive triaging, various screening modalities have been developed. These include HPV nucleic acid testing, Papanicolaou (Pap) smears, liquid-based cytology (LBC), P16/Ki-67 dual staining, HPV E6/E7 mRNA testing, DNA methylation analysis, HPV integration analysis, self-sampling, and artificial intelligence-assisted screening. Given the diversity of cervical cancer screening methods and the variations in their clinical application and scenarios, a systematic comparative analysis of the advantages and limitations of each technology is essential. This not only facilitates the accurate assessment of the suitability of different screening strategies in specific medical environments but also provides a critical theoretical basis for the optimization and innovation of future cervical cancer screening technologies.

For the sustainable development of clinical medicine, integrating the characteristics of existing screening methods and exploring more effective and cost-efficient screening protocols has become an urgent issue. There is a pressing need for innovative thinking to overcome current research bottlenecks in this field.

Literature Search Strategy: A computerized search was conducted in databases including PubMed and Web of Science, covering the period from database inception to October 2025. English search terms included "Cervical cancer," "cervical cancer screening tools," "P16/Ki67 double staining," "HPV E6/E7 mRNA testing,""PAX1 gene methylation,""HPV integration,""self-sampling,"and "Artificial intelligence." Inclusion criteria: studies related to screening methods for cervical precancerous lesions, as well as research on the development and influencing factors of cervical cancer. Exclusion criteria: literature unrelated to the theme of this article or for which the full text was unavailable.

1.1 巴氏涂片

Pap smears are a cytological screening method used to identify hyperplastic atypical cells by microscopically examining the morphology of exfoliated cervi-

cal cells. Generally, this method exhibits low sensitivity (53%–55.4%) and high specificity (84.2%–94.5%). With the advancement of medical technology, the limitations of the Pap smear in the cell collection stage have become increasingly apparent: the scope and depth of sampling are restricted, making it difficult to comprehensively and accurately collect cells from the endocervical canal and the cervical surface, which hinders subsequent morphological observation and diagnosis. Furthermore, issues with slide preparation quality (such as uneven cell distribution, cell overlapping, and obscuration), along with various factors affecting sampling, preparation, and interpretation, lead to a relatively high false-negative rate. In areas with scarce medical resources, this problem is further amplified by insufficient quality control and testing capabilities. Currently, efforts are focused on optimizing sample processing and integrating artificial intelligence with advanced detection equipment to more accurately identify abnormal cells, thereby reducing the rates of missed diagnoses and misdiagnoses. For instance, HPV testing can directly detect HPV DNA in cervical cells; it possesses high sensitivity for screening cervical precancerous lesions and cervical cancer, effectively compensating for the deficiencies of the Pap smear.

The Liquid-Based Cytology (LBC) examination method is similar to the Pap smear, but differs in its use of a specialized cell sampling brush. After sampling, the brush is quickly placed into a vial containing cell preservation solution and agitated to ensure the cells are fully dispersed in the liquid. Finally, a liquid-based cytology preparation system is used to process the sample into a thin-layer cell slide.

Research has found significant differences between LBC and Pap smear screening methods. LBC demonstrates a higher rate of satisfactory slides and a better detection rate for epithelial lesions or malignancy (89.4% compared to 80.3% for conventional Pap smears). The unsatisfactory rate for LBC slides is significantly lower than that of Pap smears (1.33% vs. 7.33%).

The detection rates for atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells where high-grade squamous intraepithelial lesion cannot be excluded (ASC-H), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL) are significantly improved compared to Pap smears, indicating higher diagnostic accuracy [**?**]. Compared to the Pap smear method, LBC is similarly characterized by simple operation and cost-effectiveness. LBC also offers advantages in precision and automation, allowing for a full display of cellular and tissue details; the liquid-based processing results in clearer images. With the development of artificial intelligence, the LBC slide reading process has achieved a higher degree of automation, which not only improves efficiency but also reduces human error, making diagnostic results more objective and accurate. Although the experience and skills of the cytotechnologist still influence the results, LBC' s automated reading systems reduce the impact of such subjectivity on diagnostic accuracy. Regarding sample collection, the cervical brushes used in LBC collect cell samples more comprehensively, improving the collection rate and integrity of the

sample while reducing the rate of cell contamination.

The false-negative rate of Pap smear screening for cervical cancer is a significant concern in Chinese general practice; the emergence of the LBC examination method has markedly reduced this rate. However, LBC requires complex processing to prepare slides from liquid samples, and its sensitivity for cervical intraepithelial neoplasia grade 2 or higher (CIN2+) is relatively low (50%-70%), which limits its value in triage diagnosis. Therefore, women diagnosed with Negative for Intraepithelial Lesion or Malignancy (NILM) via LBC usually require follow-up monitoring of HPV clearance or persistent infection status. Women who test positive for HPV 16/18 must be referred immediately for colposcopy regardless of cytological results; meanwhile, those positive for other high-risk HPV types with abnormal LBC results also require colposcopy. For women positive for other high-risk HPV types but with negative LBC results, subsequent retesting is necessary to identify persistent HPV infection, with confirmed cases referred for colposcopy. Currently, cytological testing remains the primary means of cervical cancer screening in China. However, cytology is highly subjective; different pathologists may reach divergent diagnostic results for the same slide due to differences in professional background, clinical experience, and cognitive standards for judging cell morphology. Moreover, cytological results are influenced not only by the clinician' s experience but also by sampling, slide preparation, and the patient' s age.

The sensitivity of standalone cytological testing is insufficient, leading to the possibility of missed diagnoses. Consequently, subsequent research has focused on finding a triage test that maintains high sensitivity while increasing specificity and positive predictive value, enabling the accurate identification of women at high risk for CIN grade 3 or higher (CIN3+).

A triage strategy currently exists involving P16/Ki-67, which has higher sensitivity than conventional LBC and can identify populations at high risk for CIN2+. This method also utilizes the collection of exfoliated cervical cells, with a sampling technique similar to LBC, known as P16/Ki-67 immunohistochemical testing. Studies show that dual-stain triage can increase the detection rate of precancerous lesions while reducing colposcopy referral rates and increasing confidence in women without precancerous lesions. Cost-benefit analyses indicate that dual-stain triage does not increase the resource burden. P16 and Ki-67 are proteins involved in cell cycle regulation and cell proliferation. P16 is a tumor suppressor protein expressed only during the G0 phase of the cell cycle, while Ki-67 is expressed from the G1 to S phases and is absent in the G0 phase. The $P16^{INK4a}$ (P16) gene is a tumor suppressor gene that directly participates in cell cycle regulation, known as a cyclin-dependent kinase inhibitor gene (CDKN2A). The P16 gene prevents cells from entering the S phase, thereby inhibiting cell proliferation. Inactivation of the P16 gene promotes the binding of CDK4/6 with cyclin D and subsequent phosphorylation, releasing the transcription factor E2F1, which triggers target gene transcription and the transition from G1 to S phase, promoting cell proliferation and leading to tumorigenesis. Ki-67

is a marker of cell proliferation detectable in multiple stages of the cell cycle and is an important indicator for assessing cell proliferation. Research indicates that Ki-67 expression correlates with the proliferative activity of tumor cells and can serve as a prognostic indicator for breast, lung, prostate, and cervical cancers. Furthermore, its expression levels increase progressively in precancerous and cancerous tissues, with significant differences in expression observed between normal, cervical cancer, and precancerous tissues. In normal cells, P16 and Ki-67 are not expressed simultaneously within the same cell. During oncogenic transformation, the HPV oncoprotein E7 upregulates P16, causing it to be expressed throughout the cell cycle. Therefore, the simultaneous detection of P16 and Ki-67 in the same cell indicates that the oncogenic transformation process has begun. This is currently used clinically to confirm HPV transformation in cervical biopsies for diagnosis. The staining principle involves dual staining using antibodies against these two proteins to detect the co-expression of P16/Ki-67:

Cells expressing both markers are stained brown in the cytoplasm (P16) and red in the nucleus (Ki-67). A dual-stained cell is considered positive, while single-stained cells are negative.

A prospective study in the United States validated the effectiveness of dual-stain testing for screening high-grade precancerous lesions. Dual-stain testing showed higher sensitivity than cervical cytology in screening for CIN2+ (86.7% vs. 68.5%, $P < 0.001$) with comparable specificity (95.2% vs. 95.4%, $P = 0.15$). A retrospective study showed that among 367 CIN2+ and 248 CIN3+ cases available for retrospective testing, the dual-stain method detected 258 and 182 cases, respectively (sensitivities of 70.3% and 74.9%), whereas cervical cytology detected only 190 and 126 cases (sensitivities of 51.8% and 51.9%). Research has proven that compared to Pap smears, P16/Ki-67 dual staining has significant advantages in diagnosing CIN2+ cases, offering higher sensitivity, specificity, positive predictive value, negative predictive value, and accuracy than LBC. It can reduce the missed diagnosis rate of CIN2+ in HPV-negative patients and avoid unnecessary colposcopies [**?**]. Although the dual-stain method increases screening-related and medical costs, it reduces costs associated with invasive cervical cancer. While P16/Ki-67 dual-staining technology holds significant value in cervical cancer screening, its limitations cannot be ignored. First, the technology has lower diagnostic specificity for low-grade lesions (such as CIN1), which can easily lead to overdiagnosis, increasing patient anxiety and the burden of further examinations. Second, P16/Ki-67 dual staining requires extremely high sample quality; inadequate sample collection or improper processing may lead to false-negative results, affecting diagnostic reliability.

Furthermore, the interpretation of dual-staining results is complex and requires judgment by professional pathologists; differences in the experience and expertise of pathologists may affect the consistency and accuracy of the diagnosis. Finally, the high cost of P16/Ki-67 dual staining limits its widespread application in resource-limited areas, impacting its promotion in large-scale screening.

Therefore, despite the significant advantages of P16/Ki-67 dual-staining technology in improving the accuracy of cervical cancer screening, its limitations in specificity, sample quality requirements, complexity of interpretation, and cost-effectiveness still need to be overcome through technical improvements and optimization strategies to further enhance its clinical application value.

E6/E7 mRNA

The occurrence of cervical cancer is closely related to persistent HPV infection, where mRNA transcription is a key marker of viral malignant transformation. The HPV genome primarily consists of an early gene region (E region), a late gene region, and a non-coding upstream regulatory region. As core oncogenes in the early region, the expression of E6 and E7 mRNA is a critical indicator of the HPV carcinogenic process. The E6 gene degrades the protein encoded by the tumor suppressor gene p53 through the ubiquitination pathway by binding with E6-associated protein; p53 plays a vital role in cell gene repair and cancer cell apoptosis.

The E7 protein binds to pRb, interfering with cell cycle regulation. By inhibiting specific HPV gene segments and releasing E2F factors, it promotes the transition of cells from the G1 phase to the S phase, accelerating cancer cell proliferation. Therefore, E6/E7 mRNA testing not only confirms HPV infection but, more importantly, assesses the activity of oncogenes to predict the risk of malignant transformation [**?**]. Apoptosis is an essential process for maintaining homeostasis, and the E7 protein interrupts this process by interfering with the apoptotic pathway. E6 and E7 are multifunctional proteins that cooperate and influence each other to promote viral genome amplification and continuous cell proliferation while evading immune surveillance. Additionally, E6 and E7 proteins support cell division and reduce genomic stability by affecting mechanisms such as DNA methylation and telomere maintenance. These activities collectively promote tumorigenesis. Currently, there are four methods for HPV E6/E7 mRNA detection. Due to the poor stability of single-stranded mRNA, samples in early studies were easily damaged, leading to false negatives. However, using the same cell preservation solution as LBC can improve mRNA stability. The two main detection methods currently are PreTect HPV and Aptima HPV. PreTect HPV can only detect five major HPV types that cause cervical cancer and is suitable for specific research or regions. In contrast, Aptima HPV can identify the mRNA of 14 high-risk HPV types and was the first HPV mRNA test to receive U.S. Food and Drug Administration (FDA) certification. Compared to other FDA-approved HPV testing methods, E6/E7 testing has a clear advantage in screening specificity. This test demonstrates high sensitivity and specificity in detecting high-grade lesions in cervical cytology and CIN2+. Compared to HPV DNA testing, E6/E7 testing has higher specificity and positive predictive value in cytological and histological diagnosis [**?**]. Compared to LBC, E6/E7 testing has higher sensitivity but similar specificity. Compared to P16/Ki-67, E6/E7 mRNA testing has higher sensitivity, but its positivity rate is

too high, leading to suboptimal triage performance. E6/E7 mRNA testing can serve as a triage test for cytology and HPV DNA testing, significantly reducing colposcopy referrals, though its sensitivity is slightly lower.

Its specificity and effectiveness are higher than those of HPV DNA testing, making it a supplementary method for cervical lesion screening. Women who test positive for HPV E6/E7 mRNA have a high risk of malignant progression of cervical lesions and require more care and early examination. Conversely, those who test negative can reduce unnecessary examinations and follow-ups, lowering medical costs.

Gene Methylation

Establishing an objective triage strategy that can be implemented automatically as a molecular test would have significant clinical value. Currently, DNA methylation testing targeting host genes and/or HPV genes may meet this need. Existing evidence suggests that this technology not only has higher sensitivity than LBC for detecting CIN2+ but also maintains comparable specificity.

In recent years, significant progress has been made in China in the field of screening and diagnosis of cervical cancer and its precancerous lesions, particularly regarding PAX1 gene methylation.

The PAX1 gene, as an important transcription factor, plays a key role in cell differentiation, development, and tumorigenesis. Multiple studies have shown that the methylation status of the PAX1 gene is closely related to the occurrence and development of CIN2+ and cervical cancer. As research into the methylation status of the PAX1 gene in cervical cancer and precancerous lesions deepens, it provides new ideas for early diagnosis, disease monitoring, and prognosis assessment [**?**]. The PAX1 gene is a member of the PAX gene family; the protein it encodes contains a conserved paired domain that recognizes and binds to specific DNA sequences, thereby regulating target gene transcription. PAX1 gene methylation participates in the development of cervical cancer through various pathways: it can condense the chromatin structure in the PAX1 gene promoter region, inhibiting transcription factor binding and reducing gene expression. Simultaneously, changes in methylation status may affect DNA repair enzyme activity, leading to the accumulation of DNA damage and increased genomic instability, promoting cervical cancer progression. Zhang et al. explored how the reactivation of PAX1 gene methylation silencing inhibits the proliferation and migration of cervical cancer through the WNT/TIMELESS pathway, providing a new target for cervical cancer treatment. This discovery not only enriches the molecular mechanism theory of cervical cancer but also provides a scientific basis for developing new therapeutic strategies.

Numerous studies have shown a significant increase in PAX1 gene methylation levels in cervical cancer and precancerous tissues. For example, the methylation levels of LMX1A and PAX1 genes in CIN and cervical cancer tissues are significantly higher than in normal cervical tissues and gradually increase with

the severity of the lesion. Related research suggests that methylation testing of SOX1 and PAX1 genes holds important value in the secondary triage of high-grade cervical lesions, effectively improving screening accuracy and efficiency with high sensitivity. Other studies have found changes in the promoter methylation levels of PAX1 and TP63 genes in HPV-positive patients, finding significant diagnostic value for CIN2+ lesions. Furthermore, studies by Liu and Wang Yuanpei et al. explored the clinical application of methylated PAX1 in cervical cancer screening and prognosis assessment, as well as the improved efficacy of combined PAX1 methylation and P16/Ki-67 testing in the diagnosis of atypical squamous cells. These studies enrich the theoretical system of cervical cancer screening and provide a scientific basis for practical application. Domestic scholars have also focused on the combined application of PAX1 gene methylation with other biomarkers. For instance, the hypermethylation status of JAM3/PAX1 in exfoliated cervical cells of patients with high-risk HPV infection is of great significance for the diagnosis of high-grade cervical lesions. Ji Cuihong et al. explored the triage role of SEPT9 and PAX1 methylation in women positive for HPV16 and/or HPV18, providing new ideas for precision screening. Although PAX1 gene methylation has important research and clinical value as a potential biomarker for cervical cancer and other tumors, significant limitations exist in practical application. First, insufficient specificity is a key issue.

PAX1 gene methylation occurs not only in cervical cancer but may also exist in other non-cancerous lesions or normal tissues, leading to lower specificity and affecting diagnostic accuracy. Second, the detection technology requirements are high. Currently, detection methods for PAX1 gene methylation are not fully standardized, and results may vary between different laboratories.

This affects the consistency and comparability of test results. Third, the gene methylation status is dynamic, changing over time and with disease progression; a single test may struggle to accurately reflect the disease state, necessitating multiple tests and increasing cost and complexity. In the early stages of the disease, PAX1 gene methylation levels are low and difficult to detect, leading to insufficient sensitivity for early diagnosis. Fourth, the biological significance of PAX1 gene methylation is complex. The PAX1 gene also plays an important role in normal physiological processes; changes in its methylation status may not only be a marker of tumorigenesis but could also relate to the regulation of normal physiological functions. Finally, limitations in clinical application cannot be ignored. The high cost of PAX1 gene methylation testing may limit its use in large-scale screening. In resource-limited areas, the cost of testing must be weighed against clinical benefits. In summary, although PAX1 gene methylation has potential value in tumor diagnosis, challenges remain regarding specificity, technical requirements, dynamic changes, biological complexity, and clinical application. Future research needs to further optimize detection technology and improve specificity and sensitivity for better application in clinical diagnosis and treatment.

High-risk HPV (such as HPV16 and HPV18) is closely related to the occurrence of cervical cancer. With the deepening of genomic research, scholars have gained a better understanding of the mechanisms of HPV integration and its relationship with cervical cancer. Currently, HPV integration is a novel screening tool, specifically referring to the partial or total integration of the HPV genome into the host cell chromosomes, becoming part of the host cell genome. Research shows that HPV integration is not random but tends to occur in specific hotspot regions of the host genome. In 2015, Academician Ma Ding' s team used whole-genome sequencing analysis to identify HPV integration hotspots and microhomology-mediated integration mechanisms in cervical cancer, discovering new hotspot genes such as HMGA2, DLG2, and SEMA3D. Studies have found that HPV integration affects the expression of genes like PROS1 and MIR205HG by altering DNA methylation levels. HPV16 and HPV18 are the primary integration types, but their breakpoint distributions in the HPV genome differ: HPV16 has more breakpoints in the E1 and E1^E4 regions, while HPV18 has more in the E5 region. Moreover, the whole-genome methylation level of tumor tissue is significantly lower than that of adjacent non-tumor tissue, indicating that HPV integration plays a crucial role in the development of cervical cancer [**?**]. Many studies have revealed the relationship between different HPV types and cervical lesions. Specifically, HPV16 and HPV18 are widely recognized as high-risk types. The integration of HPV DNA into the host genome is a key step in cervical carcinogenesis. Furthermore, the integration status of HPV DNA is strongly correlated with the occurrence and development of cervical cancer and the expression of tumor suppressor genes p53 and pRb. On the other hand, the loss of FHIT protein also reveals the relationship between HPV16 gene integration and cervical cancer.

HPV integration may serve as a precise risk stratification tool for cervical cancer, helping to reduce unnecessary colposcopies. For HPV16/18 positive women, HPV integration testing shows high specificity and positive predictive value; studies have shown that the HPV integration positivity rate is significantly lower than that of cytological examination. HPV integration' s specificity for CIN3+ is significantly higher than that of cytology, while its sensitivity is similar. The immediate risk for CIN3+ in HPV integration-negative cases is low; follow-up after one year showed that the progression rate to CIN3+ in HPV integration-positive women was significantly higher than in negative women. After one year of follow-up, 12.0% of women who were HPV integration-positive with baseline benign or CIN1 results developed high-grade cervical lesions. Among all conservatively managed patients with HPV integration-negative CIN2 lesions, 70% recovered spontaneously after one year. HPV status and its molecular genetic parameters also play a major role in predicting the clinical prognosis of patients with locally advanced cervical cancer. Patients who are HPV-negative or have HPV16/18 DNA in an integrated state have a poorer prognosis, while those with HPV16/18 DNA in an episomal state have a better prognosis. HPV DNA integration leads to the overexpression of E6/E7 oncogenes, increasing the resistance of tumor cells to radiotherapy and chemotherapy, thus resulting

in poorer treatment outcomes. Traditional cervical cancer screening methods suffer from insufficient coverage due to factors such as patient inconvenience, privacy concerns, and limited medical resources. Consequently, self-sampling technology has emerged. HPV testing performed on vaginal samples collected by the women themselves is called self-sampling. Currently, the WHO has included self-sampling in its guidelines as part of cervical cancer screening efforts, and the updated screening methods from the International Agency for Research on Cancer also support this recommendation. Self-sampling technology mainly includes the collection of vaginal samples, urine samples, and menstrual blood samples. The procedures are as follows: (1) For vaginal self-sampling, women can use a comfortable position such as standing, squatting, or lying down with knees bent, slowly inserting a specialized sampling brush 3–5 cm into the vagina, rotating it several times upon reaching the cervical area, and then sealing the brush in a container with preservation solution. (2) For urine self-sampling, mid-stream urine is collected into a specialized tube after cleaning the vulva, avoiding contamination by menstrual blood or secretions. (3) For menstrual blood self-sampling, methods include the dried menstrual blood spot (DMBS) method using modified sanitary pads and the liquid menstrual blood collection (LMBC) method using a dropper. The former is suitable for heavy flow, using a "Q-Pad" or modified pad with an absorbent core to collect dried blood spots. The latter is for light flow, using a funnel-shaped disposable collector and a preservation solution tube.

The principle of self-sampling primarily involves performing HR-HPV DNA testing or related biomarker testing on samples collected through various methods.

Current evidence-based medical research has fully evaluated and validated the effectiveness and feasibility of self-sampling. Systematic reviews indicate that when used to detect cervical precancerous lesions, self-sampling is comparable to traditional clinical sampling in terms of sensitivity and specificity. Simultaneously, for women who have never or not recently been screened, the self-sampling strategy significantly improves screening coverage and participation rates, showing even better results. Studies across different cultures and economic backgrounds also show good acceptability and implementation feasibility. For example, a study in Brazil reported that 79.9% of surveyed women expressed willingness to accept self-sampling and considered the procedure easy.

Numerous implementation studies, randomized controlled trials, and national screening policy evaluations both domestically and abroad show that self-sampling based on high-risk HPV testing has become an important strategy for expanding the coverage of cervical precancerous lesion screening. In high-income countries, the Netherlands integrated a mail-in self-sampling scheme into its national HPV primary screening policy, increasing the participation rate of non-responders to 1.8 times that of conventional screening. Randomized controlled trials in three Nordic countries and Australia confirmed that the screening participation rate in the self-sampling group increased by an average of 23.5% compared to the control group ($95\%CI = 18.7\%–28.3\%$). At the level

of low- and middle-income countries, cluster randomized trials showed that self-sampling increased the participation of never-screened women by 4.1 times. The WHO 2023 "Global Strategy to Accelerate the Elimination of Cervical Cancer" progress report proved its acceptability across different cultural backgrounds to be over 82%. A 2022 study in *The Lancet* showed no significant difference in the sensitivity of CIN2+ detection between self-sampling and clinical collection, making it a priority strategy for areas with insufficient medical resources.

The relative advantages of self-sampling in cervical cancer screening have been widely confirmed. First, as a primary screening tool, the detection rate of high-risk HPV and CIN2+ is comparable to conventional clinical testing. Furthermore, because self-sampling can be completed independently by women without relying on medical institutions or professionals, it can overcome geographical and human resource constraints, effectively improving screening accessibility and coverage in remote, primary-level, and resource-poor areas. Additionally, due to its privacy, ease of use, and low discomfort, it can effectively reduce the psychological barriers women face with traditional gynecological examinations, thereby significantly improving screening compliance and participation rates. Moreover, the simplified sampling process and lightweight consumables make it easy to promote on a large scale without specialized equipment, helping to control the labor and venue costs required for screening. In clinical application, those with positive self-sampling results can undergo further confirmatory tests such as liquid-based cytology; this workflow balances the sensitivity of primary screening with the specificity of confirmation, optimizing screening and triage strategies.

Especially in cases where traditional screening resource mobilization is limited, self-sampling can expand coverage at a lower cost, thereby enhancing overall screening benefits and reducing the social burden of the disease. Although self-sampling can improve participation rates, several challenges remain: the quality of self-collected samples is slightly inferior to those collected by doctors; some women refuse to use it due to privacy concerns, and variations in operational skills may affect sample quality. Implementation also faces difficulties such as sample quality control, result interpretation, continuous monitoring, and transportation issues after collection. Given regional and cultural differences, further research and policy support are required.

Artificial Intelligence-Assisted Screening

1.8.1 人工智能与细胞病理学

Cervical cytology screening is recommended for population-based screening programs. Manual review of cervical cytology smears—whether conventional smears or liquid-based preparations—is labor-intensive, prone to error, and highly dependent on the expertise of the cytopathologist. These factors can lead to reduced sensitivity and increased false-negative rates. Consequently, artificial

intelligence (AI) has begun to be applied to Pap smears and liquid-based cytology (LBC).

After years of research, the application of AI in cervical cytology screening has evolved from single-cell recognition to whole-slide intelligent identification. Early models based on Convolutional Neural Networks (CNNs) achieved preliminary identification of cell-level lesions; however, they remained limited in scenarios involving overlapping cell segmentation and complex background interference. The recent emergence of the Vision Transformer architecture has significantly enhanced feature extraction efficiency through strengthened global feature capture capabilities, performing particularly well on low-quality smears, such as those with cell stacking or uneven staining [**?**]. To address the classic challenge of overlapping cells, a fusion scheme combining Mask R-CNN and PointRend improved the Dice Similarity Coefficient (DSC) from 0.83 to 0.92. This improvement means that approximately 90% of complex cell clusters in clinical practice can be accurately segmented, laying a foundation for subsequent lesion determination. Furthermore, the introduction of unsupervised learning techniques, such as the HVS-Unsup model, has substantially reduced the cost and cycle time of algorithm training by decreasing reliance on annotated data. These models can achieve the accuracy of traditional supervised learning using only 20% of the annotated samples, which is of particular value in primary healthcare settings where accumulated data may be limited [**?**].

At the Whole Slide Imaging (WSI) analysis level, algorithm designs are becoming more aligned with actual clinical requirements. DualCytoNet, published in *Nature* in 2025, employs a two-stage "coarse screening and precise judgment" framework. It achieves high stability detection using low-cost microscopic scanning (0.2 μm/px), with an internal validation Area Under the Curve (AUC) of 0.845 and external multicenter data AUCs maintained between 0.873 and 0.891, demonstrating reliability for cross-institutional applications. Another category of algorithms, the Att-Transformer, specifically addresses the problem of "lesion sparsity." By prioritizing the screening of 200 highly suspicious cells and then aggregating their features, these algorithms increase the detection efficiency of HSIL+ by approximately 40%. This is especially suitable for primary hospitals characterized by large sample volumes and significant pressure on slide reading.

In terms of performance comparison, a 2024 meta-analysis showed that AI-assisted cervical cytology screening achieved a sensitivity of 0.98 (95% CI = 0.95–1.01) for CIN2+, which is significantly higher than the 0.94 achieved by manual screening. The specificity reached 0.98 (95% CI = 0.97–0.99), also outperforming the manual rate of 0.92. Furthermore, the comprehensive cost per case (including AI analysis, colposcopy, and biopsy) was reduced to 49 RMB, a 35% decrease compared to traditional models. This dual advantage of "high efficiency and economy" makes the promotion of AI-assisted screening more feasible in resource-limited regions.

1.8.2 人工智能与 HPV 分型分流

In the prevention and control of cervical cancer, HPV genotyping and triage serve as critical components for the early and precise identification of high-risk populations. Since 2023, the development and integration of artificial intelligence (AI) technology into this field—particularly in combination with p16/Ki-67 dual-stained cytology—has brought about a comprehensive transformation in both theoretical concepts and clinical practice. In 2021, a landmark study by Wentzensen et al. published in *JNCI* introduced the cloud-based CytoReader system. Utilizing a hybrid architecture of CNN4 and Inception-V3, the system achieved AUC values ranging from 0.74 to 0.82. The core value of this research lies in its demonstration that AI-assisted dual staining can reduce referral rates by one-third compared to traditional Pap cytology (41.9% vs. 60.1%), providing a critical foundation for the clinical implementation of this technology. By 2025, Lahrmann et al. achieved further breakthroughs with CytoReader-V2. Through a single-model design that unifies ThinPrep and SurePath slide preparation, they improved the AUC to 0.78–0.95. Furthermore, the introduction of test-time augmentation and a 30-model ensemble strategy increased the robustness of positive classifications from 67% in version V1 to 95%, significantly enhancing the model' s reliability in complex clinical scenarios.

In the context of Chinese general medicine, AI-assisted dual staining has demonstrated consistent performance gains across various populations. In a screening cohort (Kaiser, $n = 3,095$), AI-assisted dual staining achieved a specificity of 57.8%, representing a 5.2% improvement over manual dual staining (52.6%). In a referral cohort (Biopsy, $n = 409$), the specificity of AI-assisted dual staining reached 44.4%, a 9.8% increase compared to the 34.6% achieved by manual interpretation. Furthermore, in a multi-center unified population ($n = 722$), this advantage remained consistent (57.8% vs. 52.6%). these results fully demonstrate the stable incremental benefits provided by artificial intelligence across diverse clinical settings.

1.8.3 人工智能与阴道镜检测

Colposcopy serves as a critical component of the cervical cancer screening workflow, enabling direct magnified observation and targeted biopsy of cervical lesions. This process effectively enhances the detection rate and diagnostic accuracy of precancerous lesions and early-stage cancer. However, colposcopy is hindered by several limitations, including high subjectivity, constraints in equipment and infrastructure, and fluctuations in technical performance, all of which impede its widespread adoption and diagnostic efficiency in large-scale and primary care screening settings. Prior to 2021, models based on Convolutional Neural Networks (CNNs) such as ResNet and VGG primarily focused on static image classification, with AUC values maintained between 0.75 and 0.82. While these models achieved preliminary lesion identification, their ability to capture dynamic lesion features remained limited. Between 2022 and 2023, architectures combining Swin-Transformer with attention mechanisms overcame

the limitations of CNNs regarding long-range feature correlations, increasing the identification accuracy for High-grade Squamous Intraepithelial Lesions (HSIL) to 82.3% and providing a more precise quantitative basis for lesion grading. The technical breakthroughs of 2024–2025 have been particularly pivotal: the CerviCARE system achieved real-time video lesion localization at 30 fps with a sensitivity as high as 98%. Furthermore, EfficientNet-B3, optimized through quantization and pruning, achieved a latency of less than 200 ms on mobile devices, satisfying clinical real-time requirements while removing barriers for mobile applications in primary care scenarios. Research has demonstrated the high practical value of integrating artificial intelligence (AI) with colposcopy. In terms of lesion segmentation, Mask R-CNN achieved a Dice coefficient of 0.91 for pixel-level segmentation of acetic acid/iodine video frames, providing a reliable tool for quantifying lesion extent. For CIN grading, the Swin-GA-RF model utilizes single-frame RGB images.

As noted in [**?**], these advancements effectively assist clinicians in assessing the severity of lesions. In biopsy point guidance scenarios, YOLOv7-Tiny maintains a prediction error of less than 2 mm for real-time video streams.

The technology achieves a sensitivity of 94.1%, significantly improving the precision of biopsy sampling. Relevant clinical studies have demonstrated that real-time AI prompting for suspicious areas reduced the missed diagnosis rate of CIN2+ from 12% to 3%. Furthermore, precision localization technology reduced the number of required biopsy samples by an average of 1.4 pieces per case. The AI feedback mechanism has also been shown to shorten the learning curve for resident physicians by 50%, simultaneously enhancing diagnostic quality and optimizing the allocation of medical resources.

1.8.4 人工智能与新兴技术

Artificial intelligence (AI) is currently driving a paradigm shift in cervical cancer screening by integrating optics, spectroscopy, and big data. This convergence is transitioning screening methodologies from traditional laboratory settings into a new era characterized by non-invasive, real-time, and population-scale applications. The fusion of AI with emerging technologies—such as novel intrinsic fluorescence, fluorescence lifetime imaging (FLIM), Raman spectroscopy, and super-resolution endoscopy—has demonstrated significant advantages, including high pathological concordance, sensitivity, and specificity. These advancements not only build upon previous technical accumulations of AI in precision detection but also lay a robust foundation for broader clinical translation and future performance enhancements.

Furthermore, new trends in the integration of AI and cervical precancerous screening are emerging, including the development of large language models (LLMs) and gamified platforms tailored for clinical support and patient engagement. While AI has substantially improved the efficiency and accuracy of cervical cancer screening, several critical limitations persist. These include a

heavy reliance on high-quality data and the risk of algorithmic bias, a lack of model interpretability (the "black box" problem), high hardware and implementation costs, and a current deficiency in comprehensive regulatory frameworks and standardization. These challenges continue to constrain the widespread adoption and deep clinical integration of these technologies.

2 总结与展望

Cervical cancer screening methods have been in development for over half a century. Each method possesses distinct advantages and disadvantages, and clinical practitioners should select the most appropriate screening approach based on the specific circumstances of the patient. Liquid-based cytology (LBC) currently serves as the primary screening modality due to its high sensitivity and specificity; however, it is associated with higher costs. In contrast, the Pap smear remains a lower-cost alternative suitable for resource-limited regions, despite its higher false-negative rate.

Emerging screening methods, such as p16/Ki-67 dual-staining and HPV E6/E7 mRNA testing, offer significant predictive value. Nevertheless, their clinical application is currently limited by operational complexity and high costs, necessitating further research to optimize their clinical utility. In China, significant progress has been made in studying the relationship between $PAX1$ gene methylation, HPV integration, and cervical cancer or its precancerous lesions. These studies have not only revealed the potential value of these factors as molecular biomarkers but have also provided important references for the screening, diagnosis, and prognostic assessment of cervical cancer. As molecular biology techniques continue to evolve, their application in cervical cancer research is expected to become more extensive and profound. Concurrently, portable emerging detection tools, such as HPV testing strips, are under active development and are expected to provide more convenient options for cervical cancer screening.

Furthermore, it is essential to explore the deep integration of self-sampling and artificial intelligence (AI) by optimizing self-sampling protocols and AI models to enhance both the convenience and precision of screening. Future research should also investigate the interactions between HPV and other genetic variations or epigenetic modifications to fully elucidate the pathogenesis of cervical cancer. Additionally, the continuous exploration of new screening methods is required to improve sensitivity and specificity while reducing false-negative rates. These advancements must also account for economic feasibility to provide superior solutions for the prevention and early detection of cervical cancer.

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Note: Figure translations are in progress. See original paper for figures.