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Role of Blood and Urine-Based Novel Biomarkers in Cervical Cancer Detection, Screening, Prognosis: Current Advances and Future Directions

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Abstract

Cervical cancer (CC) is one of the most common cancers among women worldwide, and early detection is crucial step for increasing survival rates. Blood and urine-based biomarkers provide non-invasive, very sensitive detection of cervical cancer, allowing for earlier diagnosis and surveillance than traditional procedures such as the Pap test or visual inspection with acetic acid (VIA). These biomarkers enhance precision by detecting molecular alterations, lowering false negatives, and facilitating large-scale screening systems. This review focuses on recent advances in non-invasive biomarkers for prediction and diagnosis of cervical cancer. This review analyzes a decade of literature on blood- and urine-based noninvasive biomarkers for cervical cancer detection, screening, prognosis emphasizing recent advancements, innovative approaches, and emerging prospects in biomarker applications to enhance diagnostic precision and effectiveness.

Keywords: Cervical cancer, Non-invasive biomarkers, Blood based biomarker, Urine based biomarkers, Early detection

Table 1 Abbreviations.

Symbol	Description	Symbol	Description
CIN	Cervical Intraepithelial Neoplasia	M-CSF	Macrophage Colony Stimulating Factor
MS	Mass Spectrometry	cfDNA	Cell free DNA
SCC-Ag	Squamous Cell Carcinoma Antigen	AFD	Value Of Allele Fraction Deviation
CYFRA	Serum Fragment of Cytokeratins	PAO	Polyamine Oxidase
CEA	Carcinoembryonic Antigen	COL1A1	Collagen type I, alpha 1
HPV	Human Papillomavirus	TLBA	Reverse Line Blot Analysis
VIA	Visual Inspection with Acitic Acid	ZNF516	Zinc Finger Protein 516
CC	Cervical Cancer	VOCs	Volatile Organic Compounds
CA- 125	Cancer Antigen 125	ELISAs	Enzyme-Linked Immunosorbent Assays

Symbol	Description	Symbol	Description
VEGF-C	Vascular Endothelial Growth Factor–C	VEGF	Vascular Endothelial Growth Factor
PTR-MS	Proton transfer reaction mass spectrometry		

Introduction

Cervical cancer (CC) develops in the cervix, which is located between a woman's uterus and vagina. As per e World Health Organization's 2023 estimate, CC is the fourth most recurrent kind of carcinoma in women across the globe [1]. The Global Cancer Report states that in 2020, there were 604,127 new cases of CC diagnosed and 341,831 related deaths stated.

Cervical cancer is caused by one of the 15 HPV carcinogenic agent genotypes, which results in a persistent infection of the cervix's epithelium. The ICO Information Center reports that over 70 % of cervical cancer cases across the globe ascribed to HPV 16 and 18 types, which are also linked to 0.41 - 0.67 of high-grade cervical abnormalities and 0.16 - 0.32 of low-grade abnormalities. The progression of the disease occurs through 4 distinct stages: Initially, metaplastic changes manifest within the epithelial layer of the cervical transformation zone. Subsequently, the infection persists, exacerbating the pathological alterations. In the third stage, the disease extends beyond the epithelial layer to involve the cervix, culminating in the development of precancerous lesions. Finally, in the fourth stage, malignant cells breach the basal membrane of the epithelial layer, signifying invasive carcinoma. [3].

The HPV vaccine provides significant protection against cervical cancer; however, vaccine coverage remains suboptimal in both developing and developed nations [2,4]. Evidence suggests that women who have received the HPV vaccine should continue to undergo regular cervical screening, as no vaccine offers complete protection against the disease [5]. Thus, routine cervical screening is imperative for reducing the risk of cervical cancer, as precancerous cells typically progress to invasive cervical cancer over an approximately ten-year period [6]. Early-stage cervical cancer (stages Ib and II) has a survival rate ranging between 70 and 90 %; however, this rate plummets to approximately 15 % in regions lacking adequate screening programs [7]. The asymptomatic nature of early-stage CC complicates timely detection, contributing to higher mortality rates among affected women [8]. Early identification of precancerous lesions facilitates access to several effective therapeutic options for disease management.

In clinical practice, the Papanicolaou (Pap) test, HPV DNA testing, and colposcopy are widely used for detecting abnormal precancerous cervical cells, as shown in **Table 1**. The Pap test demonstrates a specificity of 98 % and a sensitivity of 51 % Hoskins *et al.* [9], making it particularly effective in diagnosing adenocarcinoma or CC in situ. For confirmatory diagnosis, colposcopy and cervical biopsy are commonly employed; however, these invasive methods may lead to delayed treatment decisions, increased costs, and additional risks [10]. Current screening systems have limitations, including procedural discomfort, potential embarrassment for women, invasiveness, and suboptimal sensitivity and specificity. Although various treatment options are available for CC, early detection remains crucial for improving outcomes [11]. The poor prognosis and reduced efficacy of treatments in advanced stages of CC underscore the urgent need for innovative prognostic, diagnostic, and therapeutic approaches [12].

Table 2 presents the several techniques for identifying CC, including Pap smears and blood testing, can assist differentiate between healthy and malignant cells or tissues. Conventional techniques for early-stage cancer screening frequently fall short in terms of accuracy and effectiveness, emphasizing the importance of enhanced infection detection and treatment. Biomarkers advancements have offered potent diagnostic tools for many malignancies, resulting in the creation of innovative platforms for extremely sensitive HPV detection.

Table 2 Conventional techniques of cervical cancer detection.

Conventional techniques	Advantages	Drawbacks
Pap Smear	Detects the abnormal cells in the cervix and can predict the CC at early stages; supports early diagnosis; widely recognized; high specificity.	Invasive procedure; challenging to encourage participation; requires laboratory quality assurance; limited sensitivity.
HPV DNA Testing	Accurately detects all forms of dysplasia; effective for diagnosing CC	Expensive and invasive; limited specificity.
VIA and VILI	Simple and cost-effective methods; immediate results accessibility.	Lower specificity; lack of standardization; requires specialized training for healthcare providers.
Cytology	High accuracy and specificity, often exceeding 90 %.	Invasive and painful procedure.

A cancer biomarker is a biochemical entity found in body fluids including serum, blood, or urine that enable to identify and monitor the disease presence and advancement, whether physiological or pathological [13]. Biomarkers encompass a variety of molecular and cellular entities, including proteins, peptides, enzymes, receptors, nucleic acids (DNA, miRNA), antibodies, metabolites, antigens, and other biological products [14]. Their identification improves precision medicine by allowing individuals to be stratified based on disease susceptibility, therapeutic response, and prognostic outcomes, resulting in better patient management. Biomarkers might be single molecules, such as CA-125, or more extensive proteome, genomic, or metabolomic profiles [15].

Biomarkers for cervical cancer diagnosis and progression prediction are found using a variety of methods. Although tumor biology has long been the accepted approach, new technologies like next-generation sequencing (NGS) and mass spectrometry (MS) have made it possible to find biomarkers in bodily fluids [16]. Potential biomarkers must go through crucial stages like identification, validation, and verification before they may be used in clinical settings. Thorough evaluation is necessary to guarantee analytical precision, scientific dependability, and practical utility while performing novel biomarker tests. It is anticipated that the discovery of cervical cancer biomarkers would greatly boost preventative and treatment approaches. The function of biomarkers in carcinogenesis, precancerous lesion diagnosis, and cervical cancer care has been emphasized in a number of research and reviews.

This study focuses on blood based and urine based novel non-invasive biomarkers discovered in the last decade, such as SCC Ag and different miRNAs, for diagnosing and predicting CC development. It focuses on randomized clinical trials and cohort analyses that demonstrate the critical role of these biomarkers in enhancing CC detection, screening, prognosis therapy, and overall outcomes.

Materials and methods

This article provides an explanatory overview, briefing diverse novel non-invasive CC biomarkers identified in blood, and urine. The data cited in this review spans from January 2013 to December 2024, encompassing the most recently published studies. The search process involved online databases, comprising SCOPUS, Web of Science, PubMed, and Science Direct, utilizing specific search terms such as ‘cervical cancer’, ‘biomarker’, ‘blood’, ‘urine’, ‘early detection and ‘Novel non-invasive biomarkers’.

The scope of the article is illustrated in **Figure 1**. This study reviewed a broad spectrum of literature, encompassing original research articles, review papers, systematic reviews, and meta-analyses. The focus was on novel innovative and non-invasive biomarkers identified in urine and blood, as well as those related to the detection, prognostic strategies for CC. Due to linguistic constraints, articles published in languages other than English were excluded. This review serves as

a valuable resource for understanding the pivotal role of biomarkers in CC research, particularly in detection methodologies. The inclusion of diverse studies and the implementation of a rigorous search strategy enhance the comprehensiveness of the review. It provides critical insights for researchers and healthcare professionals, facilitating the development of more effective diagnostic approaches for CC while keeping them informed of recent advancements in the field.

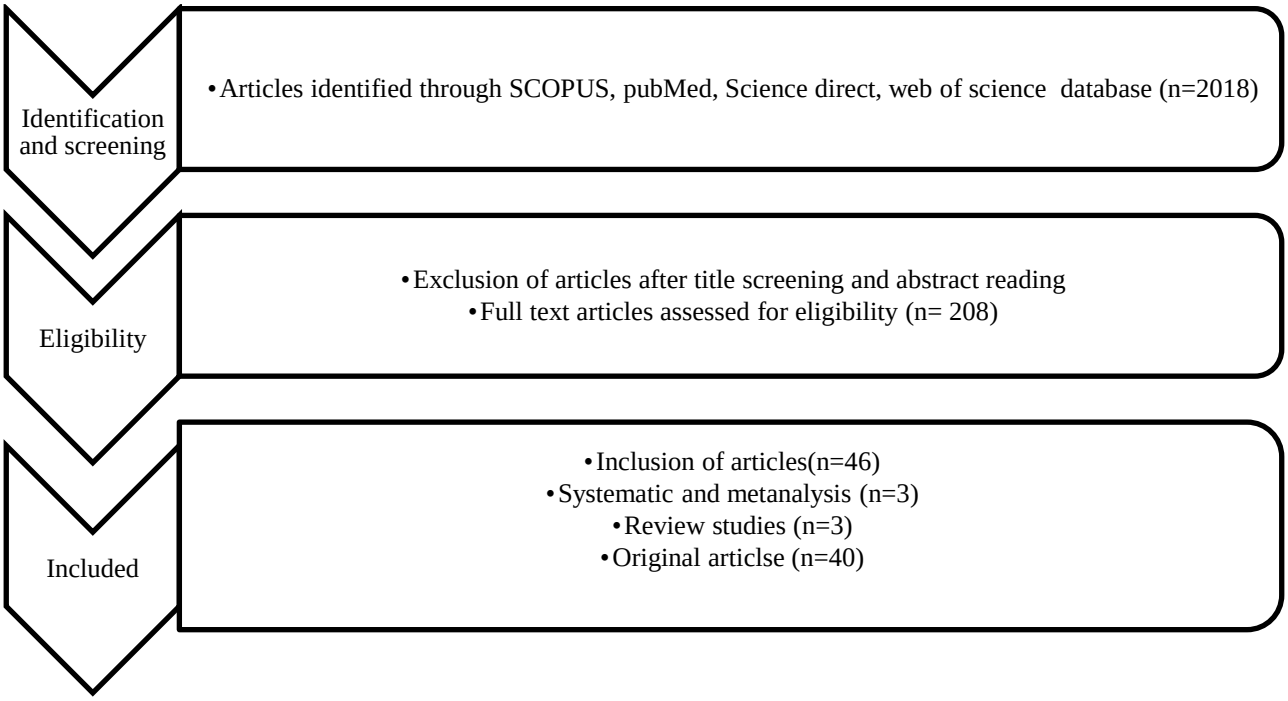

Figure 1 Diagram illustrating the process of selecting articles for evaluation.

Biomarkers and their significance in cervical cancer

The early detection of CC necessitates both precision and reliability [17]. While the Pap smear is effective in detecting squamous lesions, it is limited in identifying epithelial lesions, which require histological confirmation through biopsy. Alternatively, DNA analysis has proven valuable in identifying high-risk human papillomavirus (HPV); however, it has limitations, including low specificity in predicting inflammation-related outcomes. Biomarkers play a pivotal role at various stages of disease progression. The identification and integration of CC biomarkers facilitate early diagnosis, effectively preventing the disease from advancing to more severe stages [18]. Biomarkers support clinicians in making timely decisions regarding further diagnostic testing, treatment strategies, colposcopy referrals, intensified monitoring, or returning to routine screening protocols. Additionally, biomarkers enable the assessment of patient prognosis, the evaluation of therapeutic efficacy, and the monitoring of treatment progress. By advancing precision medicine, biomarkers allow for personalized treatment approaches tailored to individuals or specific patient subgroups based on unique biomarker profiles, thereby improving overall patient outcomes [19].

Blood-based biomarkers

Blood-based diagnostics offer a less invasive alternative compared to traditional methods such as colposcopy, Pap smear, visual inspection with acetic acid, and biopsy. A key advantage of blood-based approaches is the higher acceptance rate among women, as sample collection does not require the exposure of private body areas. Cancerous cells release specific proteins, nucleic acids, extracellular vesicles, and cellular debris into the bloodstream, which can be analyzed and detected through various advanced methodologies. The workflow of proteomic research studies designed to identify biomarkers in bodily fluids for cancer detection is illustrated in **Figure 2**. In cervical cancer, proteomic investigations are being conducted to

discover biomarkers that facilitate early diagnosis, prognosis, and the identification of therapeutic targets. These advancements underscore the potential of blood-based biomarkers in enhancing non-invasive diagnostic and precision medicine approaches.

The procedure for proteomic studies are as follows:

1) The study subjects' and controls' biological samples, such as urine, blood, tissues, and other body fluids, will be collected.

2) With the process of centrifugation, the purification of proteins is employed. The collected samples are stored at 20 to 70 °C temperatures using protease inhibitors.

3) Advanced methods highlighted in the proteomic strategy include techniques like mass spectrometry, protein microarray, Edman sequencing, 2D gel electrophoresis, and 2D-DIGE.

4) Candidate validation and database matching are conducted using tools like Western Blot, Immunohistochemistry, and ELISA followed by the creation of graphical representations.

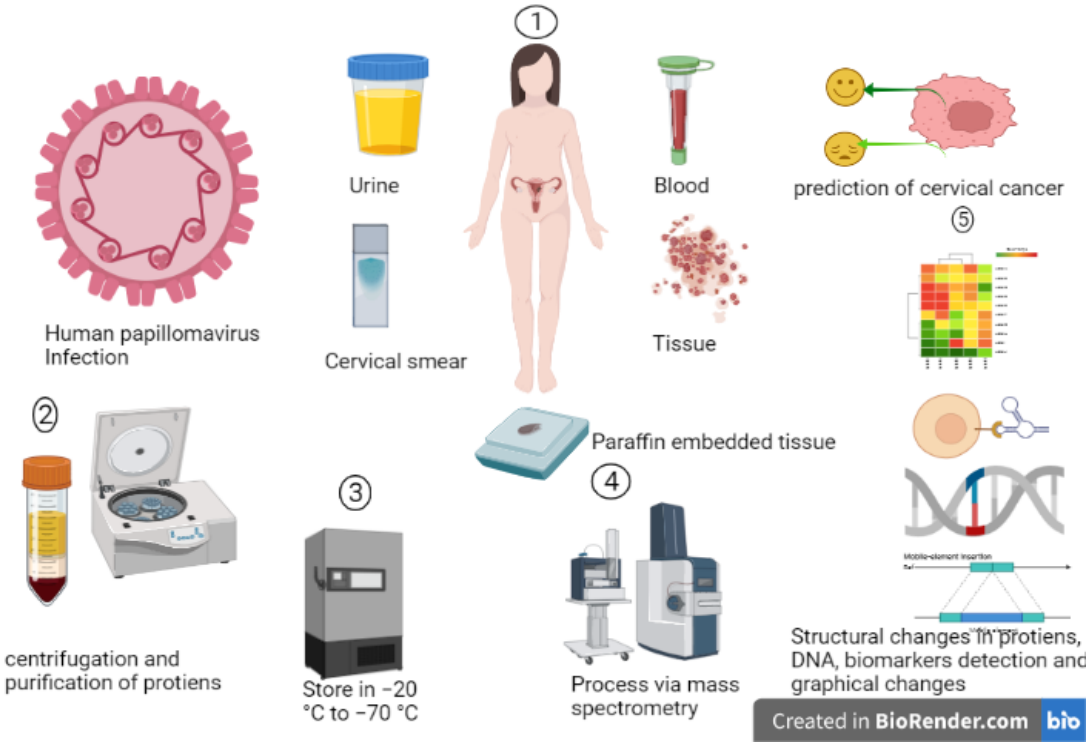

Figure 2 Workflow for cervical cancer biomarker detection and analysis.

Following are the blood-based biomarkers

Squamous cell carcinoma antigen (SCC-Ag)

SCC-Ag is a valuable serum biomarker utilized in distinguishing squamous cell carcinomas. Elevated SCC-Ag levels are observed in 20 - 60 % of patients with early-stage CC, and approximately 64 % of individuals with SCC exhibit abnormal SCC-Ag levels [20,21]. Pre-treatment SCC-Ag levels are correlated with tumor size and the stage of carcinoma [22]. A decline in SCC-Ag levels is typically noted following the initiation of treatment, while an increase may indicate disease recurrence [23]. The sensitivity and specificity of SCC-Ag for detecting CC recurrence range from 56 to 86 % and 83 to 100 %, respectively [24]. Additionally, SCC-Ag plays a vital role in managing CC patients receiving concurrent chemoradiotherapy (CCRT) or radiotherapy, as it assists in evaluating treatment effectiveness and tracking disease progression.

SCC-Ag levels aid as a valued clinical scale for determining the necessity of surgical intervention. Additionally, SCC-Ag is recognized as a highly sensitive and cost-effective biomarker for CC detection and the detection of tumor recurrence [25]. As

illustrated in **Figure 3**, cervical cancer biomarkers such as SCC-Ag play a pivotal role in facilitating diagnosis, staging, and informing therapeutic decisions. Regular monitoring of SCC-Ag levels supports the assessment of treatment efficacy and post-treatment surveillance, enabling a more comprehensive and personalized approach to patient management. Although SCC-Ag is a potential biomarker for monitoring CC progression and recurrence, its sensitivity and specificity for early-stage CC are limited, necessitating additional testing in diverse, large-scale populations to determine its utility as a standalone screening tool [24].

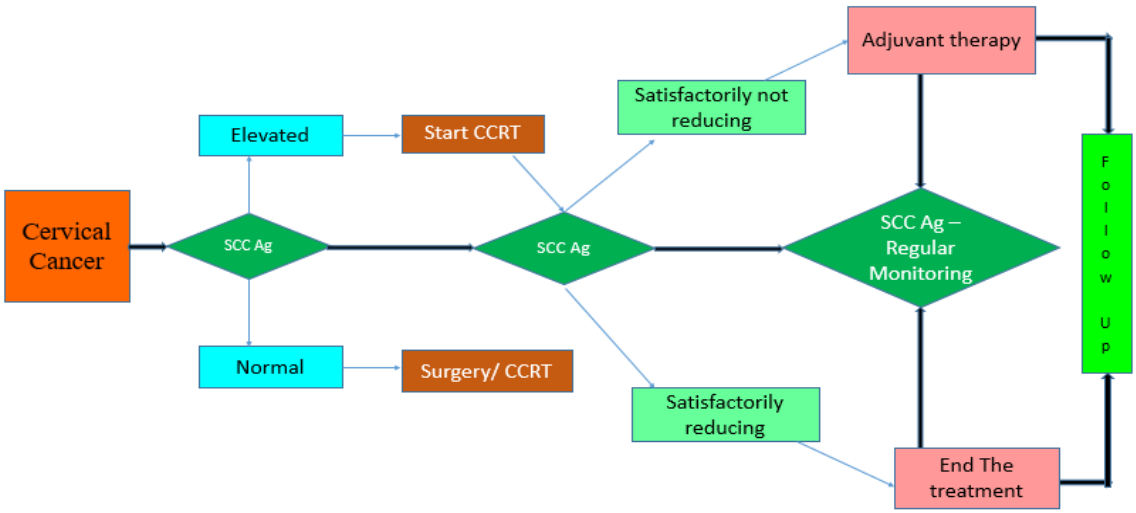

Figure 3 Role of SCC Ag detection in deciding on the treatment of women with CC.

Cancer antigen 125 (CA- 125)

Cancer Antigen 125 (CA-125) is a critical biomarker in gynecological oncology, widely utilized to monitor treatment efficacy and evaluate survival outcomes in women undergoing CC therapy [26,27]. It is detectable in both serum and vaginal secretions, with elevated levels often indicative of precancerous or malignant tumors. Increased CA-125 levels are commonly observed in advanced disease stages, with abnormal levels reported in 42.6 % of women with adenocarcinoma (ADC) and 18.9 % of those with squamous cell carcinoma (SCC). CA-125 also has prognostic value in predicting complex endometrial cancer [28,29]. Elevated levels of carcinoembryonic antigen (CEA) and CA-125 reported in cervical and uterine malignancies, with a sensitivity of 98 %. CA-125 is mainly suitable for prognostication in women with adenocarcinoma [30]. The normalization of CA-125 levels following treatment indicates a positive prognosis, while consistently high levels during or after therapy are associated with an unfavorable outcome. However, Goldberg *et al.* [31] found no significant correlation between CA-125 levels and tumor size, grade, or stage.

A study by Kim *et al.* proved that raised preoperative CA-125 levels are significantly associated with larger tumor size, parametrial extension, and lymph node metastasis in cervical adenocarcinoma patients. Specifically, patients with CA-125 levels ≥ 50 U/mL exhibited inferior 5-year locoregional recurrence-free survival (38.5 vs. 70.0 %), distant metastasis-free survival (37.0 vs. 69.4 %), and overall survival (43.6 vs. 78.1 %) matched to those with minor values [32]. Moreover, Ran *et al.* assessed the combined usage of MRI and serum tumor markers, including CA-125, for preoperative analysis of lymph node metastasis and parametrial penetration in CC. Their findings indicated that combining MRI with serum CA-125 levels improved diagnostic accuracy, with sensitivity, specificity, and accuracy rates of 76.3, 95.3, and 94.3 %, correspondingly, for lymph node metastasis detection [33]. Although studies have revealed that CA-125 levels are higher in advanced or metastatic CC women, their sensitivity and specificity for early diagnosis are low. To yet, regulatory agencies including the FDA and WHO have not authorized it as a main CC screening method. CA-125 is unsuitable for regular CC screening because to poor early-stage detection, and further study is needed

to determine its potential usage in conjunction with other biomarkers [30,31].

Cancer antigen 19-9 (CA19-9)

CA19-9 is associated with several malignancies, including hepatocellular carcinoma, pancreatic adenocarcinoma, and other epithelial-derived cancers. [34,35]. Clinically, CA19-9 has proven effective in detecting tumor recurrence in women with CC who have undergone radiation therapy [36]. study by Lin *et al*. examined the efficacy of combining cytology, hrHPV analysis, and serum CA19-9 levels in detecting cervical adenocarcinoma. The research demonstrated that integrating these diagnostic modalities enhanced the detection rates of cervical adenocarcinoma compared to using cytology or hrHPV testing alone [37]. Notably, CA19-9 levels are typically higher in women with adenocarcinoma compared to those with SCC. This biomarker serves as a critical diagnostic tool for CC, particularly in cases where CA-125 results are negative, and has been strongly correlated with cervical cancer progression [38]. The value of CA19-9 in cervical cancer is limited, and more research may be necessary.

Cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1)

CYFRA 21-1 is clinically utilized as a prognostic biomarker and is considered a valuable tool for the identification of CC, often compared to SCC-Ag. Elevated levels of CYFRA 21-1 observed in women following the initiation of treatment may indicate the presence of residual tumor tissue, suggesting disease recurrence CYFRA 21-1 has been evaluated as a tumor marker for CC [39]. A study published in Anticancer Research assessed its sensitivity in detecting cervical adenocarcinomas, finding abnormal CYFRA 21-1 values in 28 % of the 25 patients studied. The sensitivity increased to 36 % when combined with CEA and to 32 % when combined with SCCAg. Overall, at least 1 tumor marker was abnormal in 40 % of adenocarcinoma women. Higher pretreatment serum levels of CYFRA 21-1 have been associated to poorer outcomes in CC patients [40]. While studies show that high CYFRA 21-1 levels are correlated with later stages of CC, its sensitivity and specificity for early detection are inadequate, limiting its use as a main screening tool.

Vascular endothelial growth factor (VEGF)

Recent investigations have shown that IGF-II can assist with the detection of cervical cancer, whereas VEGF-C levels in plasma suggest the disease's spreading potential [30]. Elevated VEGF and VEGF-C levels are substantially related with poor CC outcomes in women. Zhou *et al.* introduced a novel detection platform using engineered filamentous phage nanofibers capable of simultaneously detecting VEGF and soluble PD-L1, demonstrating enhanced specificity and sensitivity for early detection of CC [41]. Sidorkiewicz *et al*. found significantly elevated plasma VEGF levels in CC patients, particularly in advanced stages, and emphasized its diagnostic potential, especially when combined with traditional markers like SCC-Ag and CA-125 [42]. Jovanovic *et al*. revealed that VEGF-A expression was greater in adenocarcinoma than in squamous cell carcinoma, correlating with poorer clinical outcomes and shorter survival, suggesting its utility as a prophetic biomarker [43]. Tewari *et al.* established the efficacy of VEGF-targeted therapy, showing that the adding of bevacizumab to chemotherapy better PFS and OS in advanced cervical cancer, thereby setting a new therapeutic standard [44]. Zhang *et al*. highlighted the association of VEGF with tumor aggressiveness and metastasis, identifying it as a marker of angiogenesis and a potential guide for therapeutic decisions [45]. These findings underscore VEGF's multifaceted role in enhancing cervical cancer diagnosis, prognosis, and treatment, supporting its integration into comprehensive cancer care strategies. An elevated VEGF levels have been linked to advanced stages of CC and a bad prognosis. However, its sensitivity and specificity for early diagnosis are low, making it ineffective as a solo screening biomarker.

Circulating cell-free tumour DNA

Human peripheral blood is a widely utilized source of cell-free DNA (cfDNA), serving as a biomarker with significant diagnostic, prognostic, and therapeutic applications. The cfDNA is derived from the circulatory system and can be detected in various bodily fluids, including plasma, cerebrospinal fluid, pleural fluid, urine, and saliva. Studies suggest that in healthy individuals, the hematological system is the primary source of plasma cfDNA [46]. However, in conditions such as pregnancy, cancer, organ transplantation, and

other pathological states, increased DNA release from tissues contributes to elevated cfDNA levels in the peripheral system [47]. This characteristic makes cfDNA a valuable biomarker for identifying pathological conditions without invasive procedures.

Tian *et al.* reported elevated cfDNA levels in women with CC, noting significant reductions in allele fraction deviation (AFD) following treatment. Among 22 women undergoing radiation and chemotherapy and 15 women receiving surgical interventions, a notable decrease in AFD (p = 0.029) was observed. This reduction was strongly associated with tumor size reduction in most cases. Conversely, individuals with persistent AFD exhibited greater metastatic potential, correlating with limited tumor size reduction [48]. Kang *et al.* further highlighted the diagnostic potential of HPV-related circulating cfDNA (HPV ccfDNA), identifying it in 100 % of cervical cancer patients but not in the control group. This finding underscores the specificity of HPV ccfDNA as a diagnostic biomarker in cervical cancer. These studies collectively emphasize the critical role of cfDNA and HPV ccfDNA in cervical cancer detection, therapeutic monitoring, and prognosis, reinforcing their potential in advancing non-invasive cancer diagnostics. The cfDNA has showed potential as a non-invasive diagnostic for a variety of malignancies, including cervical cancer. It has been evaluated and authorized by regulatory organizations such as the FDA for use in routine patient monitoring throughout cervical cancer therapy [49].

Circulating miRNAs

The microRNA (miRNA) genome is often altered in cancer cells and is now widely documented as a key factor in cancer advancements and development. Additionally, miRNAs act as important diagnostic and prognostic markers in CC. These small, endogenous, non-coding, single-stranded RNAs were first identified by Lee *et al*. in Caenorhabditis elegans. [50]. These molecules play a important part in controling diverse biological processes, counting cell propagation, differentiation, development, metabolism, and disease progression, through intricate regulatory mechanisms. In malignant patients, aberrantly expressed miRNAs significantly contribute to tumor initiation and progression [51].

Altered DNA methylation patterns are known to influence the expression of specific miRNAs, leading to their dysregulation in cancer [52]. A single miRNA can target thousands of genes, and collectively, miRNAs are estimated to regulate approximately one-third of the human genome Despite these insights, the precise mechanisms underlying miRNA dysregulation in cancer remain unclear. Nevertheless, the aberrant expression of miRNAs has been consistently observed across various cancer types, prominence their pivotal role in tumorigenesis and disease progression. Current study revealed that miRNAs are encapsulated within exosomes—min granules secreted into extracellular compartments such as saliva, urine, and serum. These findings underscore the possible of miRNAs as non-invasive biomarkers for cancer detection and monitoring [53]. The study of miRNAs continues to uncover their critical contributions to cancer biology, with promising implications for early detection, prognosis, and therapeutic intervention. The FDA and WHO have approved this biomarker for use in conjunction with other biomarkers [54].

HPV and miRNAs

The role of viruses in the seditious progression is significant, particularly in regulating the expression of their genes. Viral infections influence gene regulation, leading to variations in miRNA expression [55]. Persistent HPV infection has been shown to disrupt normal miRNA expression, facilitating the transformation of HPV infection into CC [56]. **Figure 4** illustrates the biomarker detection process for CC. A study investigating the relationship between the severity of intraepithelial lesions in individuals infected with HPV type-16 and the expression of 4 distinct miRNAs revealed notable findings. CC patients exhibited elevated levels of microRNA-16, microRNA-21, microRNA-34a, and microRNA-143 compared to HPV-negative women. However, no noteworthy connotation was noted between variations in the expression of microRNA-16 and microRNA-34a among patients with HSIL and HPV-negative individuals. Conversely, in HPV-negative women, microRNA-21 expression increased significantly, while microRNA-143 expression decreased [57].

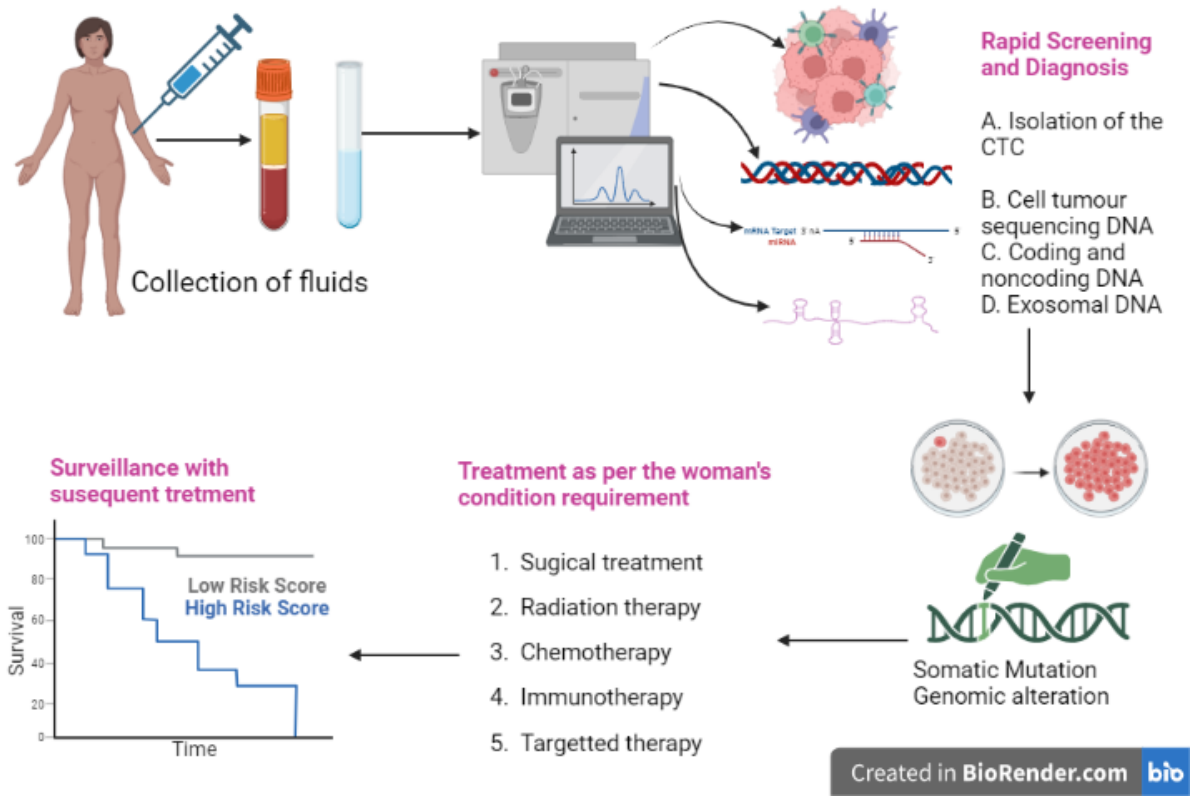

Figure 4 Workflow of biomarker-based screening, diagnosis, and personalized treatment for cervical cancer.

Nunvar *et al.* identified numerous miRNAs with aberrant expression in cervical papilloma cancers, underscoring their role in tumor development [58]. Xia *et al.* further demonstrated that aberrant miRNA expression in HPV infections is predominantly associated with viral onco-proteins such as E5, E6, and E7. These findings highlight the critical role of miRNA dysregulation in HPV-induced cervical carcinogenesis, emphasizing their potential as biomarkers for disease detection and therapeutic targets. HPV DNA testing is extensively accepted by regulatory authorities such as the FDA and the World Health Organization for cervical cancer screening. HPV DNA testing is already incorporated into regular cervical cancer screening regimens worldwide [59].

HPV-Coded miRNAs in cervical cancer

Several studies have reported that HPV viruses may encode harmful microRNAs. A bioinformatics analysis identified novel potential pre-microRNAs in various HPV strains. Notably, HPV-16 was found to possess the coding potential for 3 unique pre-microRNAs: HPV16-microRNA-1, HPV16-microRNA-2, and HPV16-microRNA-3, located in the E6, E1, and L2 open reading frames, respectively [60].

Table 3 illustrates the efficacy of putative non-invasive blood biomarkers for identifying CC. Potential biomarkers such as SCC Ag, HPV ctDNA, and microRNAs are highlighted for their roles in cancer detection and monitoring. Among these, HPV ctDNA exhibits the highest specificity (97.8 %). Additionally, serum proteins like SCC Ag and microRNAs, such as miRNA-29a, are noted for their diagnostic and prognostic relevance [61].

Table 3 An overview of performance of potential blood based non-invasive biomarkers for CC detection.

Authors	Biomarkers measured	Sample type	Test performed	Detection types (Sample size)	Sensitivity	Specificity	Current status	Key gaps
Tony *et al.* [61]	SCC Ag	Serum	PCR	30 CC patients received brachytherapy	-	-	Not mentioned	The study does not compare SCC Ag with other biomarkers.
Galati *et al.* [62]	HPV ctDNA - HPV genotyping assay	Plasma	Droplet digital PCR (ddPCR)	180 CC women and 60 were the healthy controls	86.1 %	97.8 %	Validated	The study reported that HPV16 ctDNA is less sensitive for early srage CC detection.
Du *et al.* [63]	SCCAg and MicroRNA - miRNA-29a, miRNA-25, miRNA-486-5p	Serum	Real-time PCR	140 with CC and 140 women without CC	88.60 %	92.90 %	Validated	The study only investigates SCC Ag, miRNA-29a, miRNA-25, and miRNA-486-5p, without observing into other possibly important biomarkers.
Ma *et al.* [64]	Circulating plasma microRNA	Plasma	qRT-PCR	97 with CC and 87 are controls	Not reported	Not reported	Validated	The work detects dysregulated miRNAs but does not investigate their functions and processes inCC, which limits their biological and therapeutic value.
Oh *et al.* [65]	SCC Ag to identify the relapsing CC after treatment	Serum	Serum SCC-Ag tests - immunoradiometric assay	158 with CC treated with chemo and radiation therapies	80.2 % with SCC-Ag - ≥ 2 ng/mL	94.60 %	Not validated	The study lacks an investigation of the association between SCC-Ag levels and long-term outcomes like as progression-free or overall survival, which limits its predictive practicality.
Park *et al.* [66]	miR-9, miR-21, miR-155	FFPE primary cervical and normal tissues	RT-qPCR	ervical cancer (n=52), normal tissues (n=50)	71.2 %	100 %	Not mentioned	The findings were not confirmed in an independent or external cohort, which is crucial

Authors	Biomarkers measured	Sample type	Test performed	Detection types (Sample size)	Sensitivity	Specificity	Current status	Key gaps
								for establishing their diagnostic value in various populations.
Oh *et al.* [67]	SCC Ag	Serum	Real-time PCR	53 women with repeated CC and treated with chemoradiation.	SCC-Ag: 0.49 vs. 0.887 $p < 0.001$	Not reported	Validated	The study lacks a full cost-effectiveness analysis of routine SCC-Ag monitoring, which limits its therapeutic relevance.
Ryu *et al.* [68]	SCC Ag – values in women during pre and post treatment	Serum	ICC CINtec PLUS	154 women who had readmitted with relapse of CC	Before treatment- 79.2 % After treatment - 44.2 %	Before treatment - 46.0 % After treatment - 72.0 %	Not validated	Findings lack confirmation across multiple groups, which limits their generalizability.
Tang *et al.* [69]	microRNA-218	Serum and cervical tissue	The reverse transcription -quantitative PCR	112	Not reported	Not reported	Not validated	Using hysteromyoma patients as controls may compromise the specificity of miRNA-218 results for cervical cancer.
Ma *et al.* [70]	Serum microRNA-205 for diagnostic and prognostic biomarkers	Serum	Real-time PCR	129 women (60 women with CC and 60 healthy women)	76.50 %	73.10 %	Not validate	The work lacks external validation and does not investigate the processes underlying miR-205 increase during cervical cancer development.
Kawaguchi *et al.* [71]	Squamous Cell Carcinoma antigen in post treatment	Serum	Real-time PCR	128 women with squamous cell cancer in stage IIB-IVA and treated with radiation therapy	SCC Ag more than 1.20 ng/mL 75.0 %	SCC Ag more than 1.20 ng/m 76.5 %	Not validated	The study lacks external validation of the SCC-Ag cutoff and does not look into the biological link to overall survival.

Authors	Biomarkers measured	Sample type	Test performed	Detection types (Sample size)	Sensitivity	Specificity	Current status	Key gaps
Zhao *et al.* [72]	Circulating miRNA-20a and miRNA-203 - Lymph Node Metastasis in Early Stage of CC	Blood	Real-time PCR assay	80 women with I-IIA stage of CC	Serum miR−20[a] - 75 % AUC of miR-203 - 65 %	Serum miR−20[a] were 72.5 % AUC of miR-203 - 62 %	Not validated	The work lacks external validation and does not address the mechanisms of miR-20a and miR-203 in metastasis.

Urine-based biomarker for detection of cervical cancer

Due to the increased number of invasive procedures, including Pap smear for CC detection, researchers are currently extending immense interest in detecting less invasive biomarkers. Quantitative label-free mass spectrometry is used to detect the impending biomarkers in the urine samples of women with CC. These methods differentiate the expression of urinary proteomes between healthy women and cancer. Urine is the prototype body fluid, an easily collected, non–invasive sample to discover the biomarkers due to its easy accessibility. It can be collected several times with unrestricted volumes. The urine sample collection was easy and inexpensive, with no obstacles or side effects [73].

Besides the obtainability of biomarkersin tumor tissues, serum, and blood, recent studies have revealed the presence of these markers in urine and other body fluids [74-77]. Due to the excellent homeostasis process, urine is a better noninvasive body fluid than the blood-based sample for expressing biomarkers like miRNAs [78]. Recurrently, urine samples can easily be collected in larger volumes. Though the majority of the studies were conducted to identify the biomarkers to detect CC, the collection of a urine sample could be a favorable source of mechanism as it is a less invasive procedure with a high rate of patient compliance if the reports are comparable with blood samples [79-81].

Two-dimensional gel electrophoresis, mass spectrometry (MS), and validation by immune detection are the proteomic techniques used to detect protein biomarkers in patients suffering from malignancies [82]. Recently, MS techniques have been highly used methods for the quantification of proteins as well as the identification of a more significant number of proteins [83]. This protein quantification helps enhance the vital information generated from the detected proteomes. It can compare the protein expressions among the different types of treatments used for the patients. However, label-free liquid chromatography (LCn)-MS has recently become an alternative protein quantification approach [84]. **Figure 5** displays the procedure of detection of biomarkers using urine.

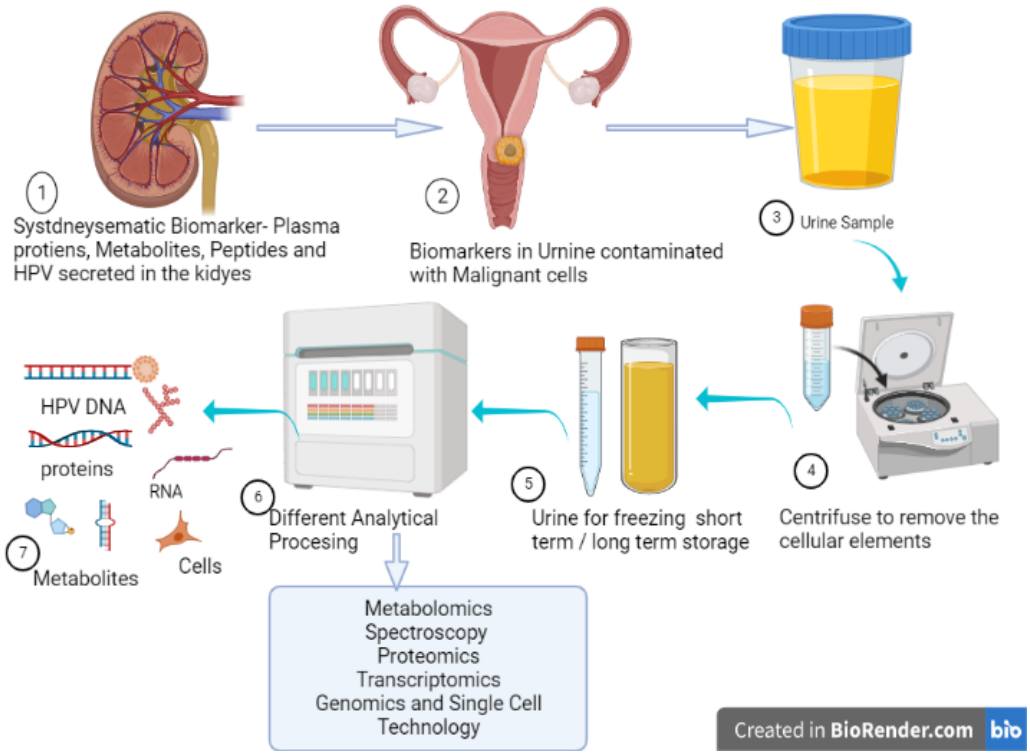

Figure 5 Process of detection of biomarkers using urine.

16α-hydroxy estrone (16α-OH E1)/2-hydroxy estrone (2-OH E1 (estrogen); ratio 5β-tetrahydro-cortisol (THF)/5α-THF

The balance of pro-carcinogenic 16α-OH E1 and protective 2-OH E1 plays a crucial role in hormone-sensitive malignancies. Elevated ratios reflect a change in pathways that promote cancer. High ratios are associated with an augmented risk of CC. Cortisol breakdown products, indicated in the THF/5α-THF ratio, have a role in inflammation and immune system modulation, which are critical in tumor growth. A 16α-OH E1 ratio above 2.5 predicted high-grade cervical lesions and cancer with a sensitivity of 85 % and specificity of 80 %. Persistent high-risk HPV infections were linked to higher 16α-OH E1 levels, suggesting a metabolic shift towards oncogenesis. Early-stage CC was shown to have a lower THF/5α-THF ratio, indicating lower activity of 5α-reductase. Altered ratios were associated with increased inflammation and immunological dysregulation, which are prevalent in tumor settings. Lower ratios were linked with progressive illness phases and lower consequences, emphasizing their possible use in prognosis and disease monitoring [85]. However, these indicators have poor sensitivity and specificity, and clinical validation is still in its early stages, necessitating more study in bigger, more varied populations. The FDA and WHO have not approved the 16α-OH E1/2-OH E1 or THF/5α-THF ratios for CCS.

Collagen I-alpha-I; collagen XVII-alpha-I

COL1A1 encodes the alpha-1 chain of type I collagen, a major structural protein in various connective tissues. Research indicates that COL1A1 expression is significantly elevated in CC tissues associated to normal tissues. This overexpression has been associated with increased resistance to radiotherapy in CC cells. Specifically, higher levels of COL1A1 correlate with reduced apoptosis following radiation treatment, suggesting that COL1A1 contributes to radioresistance in cervical cancer. Therefore, assessing COL1A1 expression could aid in predicting treatment response and possibly serve as a biomarker for cervical cancer discovery [86]. COL17A1 encodes collagen XVII, a transmembrane protein crucial for epithelial cell adhesion. Studies have shown Peptides originating from collagen XVII are detected at different levels in individuals with cervical intraepithelial neoplasia (CIN) and CC. For example, a particular peptide derived from collagen XVII showed higher

levels in the urine of CIN 2 patients compared to those with CC and was completely absent in healthy controls. This variation in expression highlights the potential of collagen XVII and its fragments as non-invasive biomarkers for the early discovery and monitoring of CC progression. [87]. While preliminary investigations indicate diagnostic promise, more validation in large, varied populations is necessary to prove sensitivity and specificity, particularly for early-stage cervical lesions. Neither COL1A1 nor COL17A1 has been approved by major regulatory authorities including FDA or WHO for cervical cancer screening.

DAPK1; RARB; twist family bHLH transcription factor 1(TWIST1; and The H-cadherin (CDH13)

COL1A1 is a primary physical constituent of the extracellular matrix, has been identified as a critical player in cervical cancer progression. Overexpression of COL1A1 contributes to increased tumor stiffness and facilitates cancer cell invasion and metastasis. Studies report that COL1A1 expression is suggestively upregulated in CC tissues associated to normal tissues. For example, immunohistochemical analyses showed that 70 - 85 % of CC samples exhibited high COL1A1 expression, correlating with advanced disease stages and poor prognosis. Elevated COL1A1 expression has been linked to radio-resistance. It was observed that patients with higher COL1A1 levels showed a 30 - 40 % reduced response to radiotherapy, potentially due to decreased apoptosis in cancer cells [88]. Collagen Type XVII Alpha 1 (COL17A1) is a transmembrane protein crucial for cell adhesion and structural integrity. Peptide levels were elevated in CIN 2 cases (up to 80 % positivity) but showed lower detection rates in advanced CC stages (approximately 30 - 40 %), indicating its potential as an early detection biomarker. These findings suggest that COL17A1-derived peptides could aid as a diagnosing tool, particularly for early-stage cervical abnormalities [89].

Zinc finger protein 516 (ZNF516); FK binding protein 6; and human papillomavirus L1 protein

ZNF516 has emerged as a promising biomarker in CC detection due to its significant differential methylation in cancerous and healthy cohorts. Promoter Methylation as a Biomarker: ZNF516 promoter methylation exhibited the highest diagnostic potential among studied markers, with a sensitivity of 90 % and specificity of 90 %. The AUC for ZNF516 was reported as 0.92, reflecting its high accuracy in distinguishing cancer cases from healthy controls. Outcomes were further validated in an incidence cohort, underscoring the reproducibility and reliability of ZNF516 as a biomarker. FKBP6, a protein involved in immune regulation and cellular processes, has shown considerable potential in cervical cancer detection through methylation analysis. The promoter methylation of FKBP6 achieved a sensitivity of 73 % and a specificity of 80 %, with an AUC of 0.80. When combined with ZNF516, the collective sensitivity and specificity of these biomarkers reached 84 and 81 %, correspondingly, making them complementary tools in CC diagnosis [90,91].

The Reverse Line Blot Analysis (TLBA) test detects the presence of HPV and enables its genotyping. The identification of HPV, particularly high-risk types, remains a cornerstone in CC detection and prevention. HPV L1 protein is a key physical constituent of the virus and a critical target in diagnostic tests. Bioinformatics analysis has been pivotal in identifying the methylation of key genes like ZNF516, FKBP6, and gamma-glutamyltransferase-like activity 4 (GGTLA4). These epigenetic changes are suggestively different in cancer patients comparing to healthy controls, providing a robust molecular basis for cancer detection [92].

Detection of microRNA in Urine as investigative and predictive biomarker

miRNAs have demonstrated significant potential as non-invasive biomarkers for CC detection. A grouping of microRNA-145-5p, microRNA-218-5p, and microRNA-34a-5p in urine showed 100 % sensitivity and 92.8 % specificity in differentiating pre-cancerous and malignant cases from placebo. These miRNAs were found to correlate with their levels in serum and tumor tissues, further validating their reliability as diagnostic tools. Furthermore, microRNA-34a-5p and microRNA-218-5p have been identified as autonomous predictive biomarkers, significantly improving survival rates for women with malignancies [93].

Multimerin 1 (MMRN1) is highly expressed in the urine of CC patients, providing a promising diagnostic target. Similarly, Leucine-Rich Alpha-2-Glycoprotein-1

(LRG1) showed a 2.72-fold increase in CC urine samples compared to non-malignant controls. Importantly, LRG1 is detectable in urine at earlier stages, while in serum, it is found only in advanced stages (II-IV). This highlights its possible as a non-invasive biomarker for early CC detection [61]. CD44, a transmembrane protein associated with cell adhesion and metastasis, has distinct expression patterns in CC stages. Higher expression of CD44 was observed in stage I cervical cancer and control cases, whereas its expression was reduced in stages II-IV. Additionally, CD44 has been detected in bladder carcinoma urine samples, emphasizing its broader utility in cancer detection [94].

Table 4 An overview of performance of potential ursine based non-invasive biomarkers for CC detection.

Authors	Biomarkers measured	Sample type	Test performed	(Sample size)	Sensitivity	Specificity	Current status	Key gaps
An *et al.* [95]	Cysteine	Urine	Molecular probe NPO-B	1,500	-	-	Validated	The novel urine-based technique shows promise, but further clinical trials are needed to demonstrate its accuracy and applicability in real-world scenarios.
Aftab *et al.* [96]	miRNA	Urine	-	Urine of pre-cancerous lesion and healthy women	100 %	92.8 %	Validated	There is limited study on urine miRNAs as non-invasive indicators for cervical cancer. Larger investigations are required to evaluate the accuracy and clinical utility of these biomarkers.
Oliveira *et al.* [97]	HPV E6 oncoprotein	Paired urine, and cervical scrap			Sensitivity for Urine: 50.0 % ($p < 0.01$)	-	Validated	Urine-based and self-collected samples show low sensitivity for detecting CIN2/3 lesions, necessitating further refinement and standardization of protocols to improve accuracy

Authors	Biomarkers measured	Sample type	Test performed	(Sample size)	Sensitivity	Specificity	Current status	Key gaps
Snoek *et al.* [98]	DNA methylation,	Urine and cervical scraps	-	Urine sample 41 and cervical scraps 38	0.744 – 0.887	-	Validated	Urine-based DNA methylation testing for CC diagnosis requires additional large-scale validation to ensure accuracy and dependability when compared to conventional cervical samples.
Leeman *et al.* [99]	HPV16/18 genotyping, RNA, and proteins	Urine	HPV test	113 women with abnormal Pap test.	95 % in all samples	Not reported	Not validated	No clear advantage of first-void urine (U1) over later samples (U2) in HPV testing.
Cuzick *et al.* [100]	Human papillomavirus (HPV)	Urine	Trovagene HPV test	501	CIN3+(n=145) 96.3 % IN2+ (n = 81) 94.5 % urine-CIN3+ 91.4 and IN2+88.3 %	< CIN2 was similar: 24.7 %		The Trovagene HPV test has somewhat lower sensitivity in urine than in cervical samples and requires additional development.
Sahasrabuddhe *et al.* [101]	E1 region of the HPV genome-cell-free HPV DNA	Urine	Trovagene HPV test	72 women referred to colposcopy	CIN2/3, 80.8 % CIN3-90.0 %,	36 % for CIN2/3 and 28 % for CIN3	Not validated	The Trovagene HPV test's poor specificity needs improvement to increase diagnosis accuracy.
Chokchaichamnankit *et al.* [102]	LRG1 MMRN1, CD44, S100A8 SERPINB3	Urine	Label-free mass spectrometry	-	100 % (AUC = 0.993) 83.3 % (0.87) 88.9 % (0.938) 100 % (0.986) 100 (1.000)	87.5 % 87.5 % 87.5 % 87.5 % 87.5 %	Validated	The molecular functions of the discovered biomarkers in cervical cancer development are unknown, which limits their usefulness as therapeutic targets.

Authors	Biomarkers measured	Sample type	Test performed	(Sample size)	Sensitivity	Specificity	Current status	Key gaps
Senkomago *et al.* [103]	HR-HPV DNA HR-HPV mRNA-	Urine	Trovagene test. HR-HPV mRNA-Aptima HPV assay	37	89.90 %	Not reported	Not reported	The study's findings are limited by the small sample size, necessitating validation in larger, more diverse populations to establish the efficacy of urine-based HR-HPV testing for detecting CIN2+.
Garbett *et al*. [104]	Collagen I-alpha-I; collagen XVII-alpha-I (protein class)	Urine	Amicon Ultra-4 (10,000 NMWCO) of supernatant!	biopsy confirmed; CIN2 (n=4); cervical cancer (n=4); controls (n=4)	No report	Not reported	Not validated	DSC patterns show plasma protein interactions, although precise biomarkers and processes are unknown.

Discussion

Several non-invasive diagnostic measures have been advanced in recent decades, efforts have been made to address the limitations of invasive procedures and improve the accuracy of diagnostic outcomes in CC. Biomarkers found in blood, serum, and other bodily fluids play a crucial role in diagnosing CC. Although blood is the primary source of biomarkers for cancer detection, other promising indicators have been identified in urine, cervical smears, tears, breath, and hair. According to the current review, if women test positive despite normal cervical cytological tests such as Pap test, VIA, or VILI, the following biomarkers tests are suggested for the confirmation of CC. These tests can be performed using blood or urine as body fluids and include: SCC-Ag, CA-125, CA19-9, CYFRA 21-1, VEGF, Circulating Cell-Free Tumor DNA, circulating miRNAs, HPV and HPV-encoded miRNAs, 16α-OH E1/ 2-OH E1 (estrogen) ratio, 5β-tetrahydrocortisol (THF)/5α-THF, Collagen I-alpha-I, Collagen XVII-alpha-I, DAPK1, RARB, Twist Family bHLH Transcription Factor 1 (TWIST1), H-cadherin (CDH13), Zinc Finger Protein 516 (ZNF516), FK Binding Protein 6, and the Human Papillomavirus L1 Protein.

According to recent studies findings, exposure to HPV E6/E7 mRNA increases the probability of developing high-grade CC because viral DNA integration disrupts the E2 tumor suppressor gene, resulting in E6 and E7 oncogene overexpression [105]. HPV E6/E7 mRNA tests have analytic usefulness for CIN2+, with sensitivity, specificity, PPV, NPV, and AUC ranging from 0.65 - 0.99, 0.42 - 0.9, 0.1 - 0.85, 0.66 - 0.99, and 59 - 80 %, correspondingly [106]. Zhu *et al*. reported that variability in study populations causes variances in specificity from 42.7 to 90.2 %, as well as PPV from 10 to 85.9 % [107].

Meanwhile, SCC-Ag is generally measured in blood, although urine has lately been investigated as an alternate source for detecting SCC-Ag. SCC-Ag has a diagnostic performance and sensitivity ranging from 56 to 86 %, depending on the illness stage, and a specificity of 83 to 100 %. Singh *et al*. found that regular monitoring of SCC-Ag levels is an effective diagnostic

approach for detecting recurrences at an early stage, even beforehand visual indicators appear, with a sensitivity of 0.90 and a specificity of 0.92 [108]. Monitoring SCC-Ag is especially important for identifying tumor recurrence following concurrent chemoradiation treatment (CCRT). However, investigations have yielded conflicting results regarding the diagnostic and prognostic significance of SCC-Ag. To overcome this, standardized scoring methods and detection procedures based on SCC-Ag should be created to achieve consistent and comparable findings while limiting variances between clinical investigations [109].

Moreover, our review of the diagnostic recital of miRNA tests, namely miR-9, in identifying CIN2+ found sensitivity, specificity, and AUC values ranging from 52.9 - 67.3, 76.4 - 94.4, and 0.71 - 0.85 %, respectively [110]. Furthermore, Park *et al.* found PPV of 77.7 % and a NPV of 70.2 % for miR-9 tests. The remarkable specificity obtained emphasizes miR-9's diagnostic importance in diagnosing CIN2+. MiR-9, like other circulating miRNAs, has been demonstrated in studies to have an important role in initial discovery of CC prognosis prediction, and clinical outcome tracking [111,112]. Furthermore, miR-9 levels in exfoliated cells, cervical tissues, or serum, as well as their relationship with biological processes such as metabolism and apoptosis, have consistently demonstrated their purposes in assessing the risk of CIN in alleged cases [113]. This value is especially obvious when miR-9 is paired with additional markers including miR-21, miR-155, miR-192, miR-203, and miR-205, which improves specificity and optimizes therapeutic techniques [114].

In addition, DNA methylation assays demonstrated variable performance in detecting CIN2+, with sensitivity extending from 59 to 92.9 %, specificity from 67 to 98 %, PPV from 15 to 95.4 %, NPV from 65.5 to 98.3 %, and AUC between 0.81 and 0.86 [23,38-44]. Schmitz *et al.* [115] reported the lowest sensitivity of 59.7 %, whereas Dong *et al.* reported the highest at 92.9 % [117]. DNA methylation levels are increased in CIN2+ and CIN3+ instances, hence gene methylation analysis in cytology samples is a promising triage tool for HPV-reactive patients. This approach has higher specificity than ASCUS cytology and greater sensitivity than HPV16/18 genotyping. Liquid-based cytology (LBC) sample is convenient and has quicker turnaround times, which promote its usage in settings lacking histological infrastructure. However, bigger, more varied cohort studies are required to confirm its therapeutic value.

VEGF assays reviewed in present review showed sensitivity, specificity, and AUC values ranging from 0.56 - 0.83, 0.74 - 0.96, and 83 - 86 %, correspondingly [123-126], with Lawicki *et al.* [124] reporting a PPV of 0.86 and a NPV of 0. 82 %. These findings align with earlier suggestions by Sidorkiewicz *et al*. and Cheng *et al*., highlighting the analytical relevance and clinical practice of VEGF assays in cancers such as cervical, breast, and endometrial cancer. The consistency in specificity and AUC across studies, along with its diagnostic correlation with complementary markers like M-CSF and SCC-Ag, reinforces its usage. Additionally, Ceci *et al.* noted that VEGF overexpression is often associated with larger tumor size, pelvic lymph node participation, and parametrial penetration [127], a conclusion also reinforced by Zusterzeel *et al.* [128]. Consequently, our findings confirm the clinical potential of VEGF in diagnosing cervical cancer, although further large-scale clinical translation studies with diverse cohorts are essential to validate these observations.

As researchers continue to unravel the complexities of these novel biomarkers, it becomes evident that leveraging non-invasive indicators found in various physiological fluids represents the future of cervical cancer detection. This review highlights the current state of research and serves as a roadmap for future studies, fostering collaboration and innovation in the pursuit of more effective, patient-friendly approaches to cervical cancer prediction and diagnosis. These advancements hold the potential to improve outcomes, promote early intervention, and contribute significantly to global cervical cancer prevention efforts.

This study emphasizes the need for future research focusing on biomarkers found in blood and urine, for detecting CC. The non-invasive nature and ease of sample collection from these bodily fluids make this approach particularly promising for enhancing cervical cancer detection. The convenience and comfort of non-invasive sampling lead to higher screening rates, aid in early detection, and improve overall success in

combating CC. Thus, researchers should prioritize and conduct further studies to evaluate and validate the use of biomarker detection in these accessible physiological fluids, paving the way for more effective and patient-centered diagnostic strategies.

Conclusions

The paper presents substantial advancements in cervical cancer detection using non-invasive biomarkers taken from blood and urine. These biomarkers, including SCC-Ag, CA-125, and miRNAs, have a strong potential for enhancing diagnostic accuracy and early disease identification. Blood-based biomarkers, including SCC-Ag and CA-125, have a strong potential for enhancing diagnostic accuracy and early disease identification that are frequently overlooked by traditional methods such as Pap test and HPV DNA assays. Similarly, urine-based biomarkers comprising miRNA-34a-5p, MMRN1, and LRG1 provide cost-effective, patient-friendly alternatives, which are especially useful in low resource health care settings where invasive treatments are challenging to adopt. These biomarkers improve diagnostic accuracy by lowering false positives and false negatives using modern techniques like as proteomics and epigenetic profiling. Furthermore, biomarkers such as cfDNA and circulating miRNAs enable real-time monitoring of therapy effectiveness and illness recurrence, filling a gap in dynamic disease tracking that standard static techniques fail to address. Communally, these innovations reassure early detection, customized cancer therapies, and increased accessibility, addressing critical gaps in current cervical cancer screening and diagnostic frameworks.

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