RESEARCH ARTICLE

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Diagnostic Performance of Serum VEGF and M-CSF in Cervical Cancer and, Their Association with Stage

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Abstract

Background: Cervical cancer continues to be one of the most prevalent malignancies affecting women globally. Circulating biomarkers may provide added value by complementing HPV-based screening and enhancing risk stratification. The aim of this study was to investigate vascular endothelial growth factor (VEGF) and macrophage colony-stimulating factor (M-CSF) as candidate serum biomarkers for the diagnosis and disease monitoring of cervical cancer. **Methods:** In a single-center case–control study, 45 women with cervical cancer and 45 healthy controls were enrolled. Clinical variables included FIGO 2018 stage, symptoms, smoking status, and HPV vaccination status. Serum VEGF and M-CSF levels were measured using ELISA. Group and stage differences were assessed, and diagnostic performance was evaluated using ROC analysis, Youden's index, and logistic regression. **Results:** Both biomarkers were significantly elevated in cancer patients compared with controls (M-CSF: 1457 ± 582 vs. 504 ± 250 pg/mL; VEGF: 399.7 ± 136 vs. 106.7 ± 53.2 pg/mL; both $p < 0.001$). Concentrations increased with advancing stage (M-CSF: $p = 0.0006$; VEGF: $p = 0.0073$). Vaccinated patients exhibited lower VEGF levels ($p = 0.047$). Diagnostic performance was excellent (AUC: M-CSF, 0.95; VEGF, 0.97). At optimal cut-offs, VEGF achieved 95% sensitivity (95% CI: 83.1%–99.4%) and 93% specificity (95% CI: 81.3%–98.5%). The combined model (AUC: 0.974) outperformed M-CSF alone ($p = 0.038$). Multivariate analysis confirmed that both VEGF and M-CSF remained significant independent predictors after adjusting for age, smoking status, and HPV vaccination status. **Conclusions:** Serum VEGF and M-CSF demonstrate diagnostic utility and stage association in cervical cancer. Their combined use enhances discriminatory power. These findings support their potential as adjunct biomarkers; however, external validation and longitudinal studies are needed.

Keywords: Cervical cancer- Human papillomavirus (HPV)- Vascular endothelial growth factor (VEGF)

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Introduction

Cervical cancer remains a major public-health challenge despite being largely preventable. In 2022, an estimated 660,000 women were diagnosed and 350,000 died from the disease, with the greatest burden observed in low- and middle-income countries (LMICs) [1]. Persistent infection with high-risk human papillomavirus (HPV) is the necessary cause of almost all cervical cancers, making HPV prevention and screening the cornerstone of control strategies [2]. In 2021, the World Health Organization (WHO) recommended HPV DNA testing as the primary screening method and launched an elimination strategy emphasizing vaccination, high-performance screening, and timely treatment [3, 4] . National vaccination programs, such as those in England, have already demonstrated substantial reductions in cervical cancer incidence, although coverage gaps remain [5, 6].

While HPV-based screening is highly sensitive, there is a parallel clinical need for minimally invasive blood biomarkers that can (i) aid triage when diagnostic work-up is equivocal, (ii) reflect tumor biology for risk stratification, and (iii) be repeated during follow-up for monitoring. Two promising candidates are vascular endothelial growth factor (VEGF) a master regulator of angiogenesis and macrophage colony-stimulating factor (M-CSF)a key driver of monocyte recruitment and tumor-associated macrophage (TAM) polarization. Elevated pre-treatment serum VEGF has been associated with higher stage and poorer outcomes in cervical cancer, and the clinical relevance of VEGF signaling is underscored by survival gains with bevacizumab (anti-VEGF-A) in

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advanced disease (GOG-240) [7-9]. M-CSF/CSF1R signaling shapes an immunosuppressive, pro-angiogenic microenvironment via TAMs; contemporary reviews and meta-analyses link higher TAM density with adverse clinicopathologic features and prognosis in cervical cancer [10, 11].

Empirically, circulating VEGF and M-CSF are elevated in cervical cancer versus controls, and early studies suggested meaningful receiver operating characteristic (ROC) performance especially when combined with traditional markers. For example, a BMC Cancer cohort found both plasma VEGF and M-CSF increased in cancer and contributed to diagnostic models, while a systematic review estimated pooled diagnostic accuracy for M-CSF alone (summary area under the curve [AUC] ≈ 0.86, sensitivity ~70%, specificity ~84%) [12, 13]. Mechanistically, HPV E6/E7 oncoproteins enhance hypoxia-responsive transcription (HIF-1α) and can upregulate VEGF, providing a plausible viral–angiogenesis axis that could be mirrored in serum [14].

Knowledge gaps remain where most prior reports evaluated single markers, used heterogeneous assays, or lacked multivariable combinations optimized on the same study. Few studies mapped stage-wise gradients for both markers simultaneously or explored patient factors such as prior HPV vaccination that might modulate systemic angiogenic or myeloid signals. Addressing these gaps could clarify whether VEGF and M-CSF provide complementary information about angiogenesis and myeloid remodeling with potential clinical utility beyond screening.

Study objective: This case–control study was designed to quantify serum VEGF and M-CSF levels in cervical cancer patients and healthy controls, compare biomarker concentrations across FIGO 2018 stages, and assess their diagnostic performance individually and in combination. Associations with HPV vaccination among patients were also investigated. The simultaneous head-to-head evaluation of VEGF and M-CSF, together with stage-stratified analyses and multivariable ROC modeling, constitutes the central focus of this work.

Materials and Methods

Study Design and Oversight

A single-center, case–control study was conducted between September 10, 2024, and July 12, 2025. involving women with histologically confirmed cervical cancer and age-matched healthy controls. Methods and reporting followed the STROBE statement for observational studies [15]. The study was approved by the Al-Qadisiyah General Hospital, Al-Diwaniyah Governorate, Iraq, with the ethical approval number M240906, dated September 2, 2024, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Study Timeline: The study commenced on September 10, 2024, following ethical approval. The main recruitment period for the 90 participants was from September 2024 to December 2024. Laboratory analysis was conducted from January 2025 to March 2025, and data analysis was completed by July 12, 2025.

Participants

Eligible cases were ≥18 years old with newly diagnosed cervical cancer and no prior oncologic therapy at the time of sampling. Disease stage was determined by gynecologic oncologists using the 2018 FIGO system, which integrates clinical examination with imaging and pathology when available [16]. Healthy controls were recruited from a well-woman clinic and had no history of malignancy or active inflammatory/infectious conditions. Pregnant women and individuals with acute systemic inflammation were excluded to minimize biomarker confounding. In total, 45 cases and 45 controls were enrolled.

Control Group Details: Controls were recruited from the comprehensive well-woman clinic at Al-Qadisiyah General Hospital. All controls had a negative Pap Smear and were negative for high-risk HPV DNA via PCR testing. Frequency matching was used to ensure a similar age distribution between cases and controls.

Clinical Variables and Definitions

Age at enrollment was abstracted from medical records. HPV vaccination status was recorded from immunization documents when available or from patient report;participants were considered "vaccinated" if they had received ≥1 dose of any HPV vaccine. Smoking status was recorded as smoker vs. non-smoker. Presenting symptoms were documented on a structured form (vaginal bleeding, dyspareunia, pelvic pain, or none). Stage was assigned according to FIGO 2018 criteria [16]. The histological type and tumor grade were documented for all cases (Table 8). HPV genotype data was not collected in this study.

Specimen Collection and Processing

Venous blood was drawn by trained phlebotomists following CLSI GP41 and WHO Best Practices in Phlebotomy [17].Samples were collected into serum separator tubes, allowed to clot for ~30 minutes, centrifuged at 1,300–2,000 × g for 10–15 minutes, and serum was aliquoted into polypropylene vials. Aliquots were stored at −80 °C, and a single freeze–thaw cycle was allowed to reduce pre-analytical variability. Because VEGF is sensitive to handling (matrix, clotting, freeze–thaw), these steps were standardized [18, 19].

Biomarker Quantification (ELISA)

Serum VEGF-A and M-CSF were measured using commercial Primacu™ ELISA kits (BT LAB, Shanghai, China).VEGF was quantified with kit BPE007 (sensitivity 3.38 pg/mL; detection range 15.63–1000 pg/mL), and M-CSF with kit BPE226 (sensitivity 9.41 pg/mL; detection range 23.44–1500 pg/mL).Assays were performed in duplicate per the manufacturer's instructions. Concentrations were calculated from eight-point standard curves using a four-parameter logistic (4-PL) regression model. Results with a coefficient of variation (CV) >15% were re-assayed to ensure quality control.

Outcomes

Primary outcomes were serum concentrations of VEGF and M-CSF and their diagnostic performance for

distinguishing cervical cancer from controls. Secondary outcomes were stage-wise gradients (FIGO I–IV) and exploratory associations with HPV vaccination among cases.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9, with continuous variables expressed as mean ± standard deviation and categorical data as frequencies (percentages); group comparisons utilized unpaired t-tests or chi-square tests, while differences across FIGO stages were assessed via one-way ANOVA with Tukey's post-hoc test, and diagnostic performance was evaluated using ROC analysis (AUC, 95% CI) with optimal cut-offs determined by Youden's index, logistic regression models were compared via DeLong's test and AIC. Multivariate logistic regression models were used to assess the independent predictive value of the biomarkers after adjusting for age, smoking, and HPV vaccination status (Table 7). A two-sided p-value < 0.05 defined statistical significance.

Sample Size Rationale

We planned equal case–control numbers to stabilize ROC estimates. Using diagnostic-accuracy planning formulae (Hanley–McNeil and Obuchowski for AUC precision, DeLong for paired-AUC comparisons), a sample of ~40–45 per group provides ≥80% power to detect an AUC in the 0.85–0.90 range versus 0.5, with 95% CI half-widths of ~0.06–0.08. This also yields acceptable precision for sensitivity/specificity estimates at ROC-derived cut-offs (Buderer's method).Guided by these considerations and recruitment feasibility, a total of n = 90 (45 cases and 45 controls) was targeted and achieved it [20-22].

Results

Demographic and Clinical Characteristics and Biomarker Levels

Comparison between the 45 cervical cancer cases and 45 healthy controls showed that the groups were comparable in mean age (p = 0.5). However, significant disparities were observed in HPV vaccination status (35% vs. 69%, p = 0.015) and smoking status (67% vs. 38%, p = 0.014). Presenting symptoms were notably more common in cancer patients.

Serum Biomarker Levels: Biomarker analysis revealed significantly elevated levels of both M-CSF (1457 ± 582 pg/mL vs. 504 ± 250 pg/mL, p < 0.001) and VEGF (399.7 ± 136 pg/mL vs. 106.7 ± 53.2 pg/mL, p < 0.001) in the cancer group compared to healthy controls (Table 1).

Association with Clinical Stage

Both M-CSF and VEGF levels showed a progressive and statistically significant increase with disease progression across FIGO 2018 stages (I-IV) (Table 2). M-CSF levels rose from Stage I (1102 ± 414.2 pg/mL) to Stage IV (2161 ± 286 pg/mL). Similarly, VEGF levels increased from Stage I (297 ± 99.3 pg/mL) to Stage IV (493 ± 81.9 pg/mL). One-Way ANOVA revealed highly significant differences across stages for both M-CSF (p = 0.0006) and VEGF (p = 0.0073).

Symptom Distribution by Stage

Vaginal bleeding was the most common symptom overall, reported in 33.3% of Stage I, 37.5% of Stage II, 27.3% of Stage III, and 50.0% of Stage IV patients (Table 3).

Diagnostic Performance of Biomarkers

Both M-CSF and VEGF demonstrated excellent diagnostic performance (Table 4 and Figure 1). VEGF

Table 1. Comparison of Demographic Characteristics and Biomarker Profiles Between Healthy Controls and Cervical Cancer Patients

Demographic & biomarker features		Healthy Groups n = 45	Cervical Cancer n = 45	p-value
Age (Years)	Mean ±SD	48.9 ± 11.4	46.5 ± 11.3	0.5[a]
HPV Vaccine Status	Vaccinated n (%)	31 (69%)	16 (35%)	0.015[b]*
	Not Vaccinated n (%)	14 (31%)	29 (65%)	
Smoking Status	Yes n (%)	17 (38%)	30 (67 %)	0.014[b]*
	No n (%)	28 (62%)	15 (33 %)	
Presenting Symptoms, n (%)	Bleeding	16 (35.6%)	0 (0.0%)	<0.001[b] ***
	Dyspareunia	12 (26.7%)	0 (0.0%)	
	Pelvic Pain	13 (28.9%)	5 (11.0%)	
	None	4 (8.9%)	40 (89.0%)	
Cancer Stage Distribution	Stage I	--	12 (26.7 %)	--
	Stage II	--	16 (35.6%)	
	Stage III	--	11 (24.4%)	
	Stage IV	--	6 (13.3%)	
M-CSF (pg/mL)	Mean ±SD	504 ± 250	1457 ± 582	<0.001[a] ***
VEGF (pg/mL)	Mean ±SD	106.7 ± 53.2	399.7 ± 136	<0.001[a] ***

n, number of cases; SD, standard deviation; ** P <0.00, statistical significance. a, Independent sample t-test; b, Chi-square. NS: Not Significant

Table 2. Serum M-CSF and VEGF Levels Across Cervical Cancer Stages

Parameter	Stage I (n=12)	Stage II (n=16)	Stage III (n=11)	Stage IV (n=6)	p-value
M-CSF (pg/mL)	1102 ± 414.2[A]	1496 ± 564.2[B]	1334 ± 544.8[B]	2161 ± 286[C]	0.0006
VEGF (pg/mL)	297 ± 99.3[A]	417 ± 123.6[B]	442 ± 158[B]	493 ± 81.9[B]	0.0073

One-Way ANOVA

Table 3. Distribution of Presenting Symptoms by Cancer Stage in Cervical Cancer Patients

Symptom	Stage I (n=12)	Stage II (n=16)	Stage III (n=11)	Stage IV (n=6)
Bleeding	4 (33.3%)	6 (37.5%)	3 (27.3%)	3 (50.0%)
Dyspareunia	3 (25.0%)	4 (25.0%)	4 (36.4%)	1 (16.7%)
Pelvic Pain	4 (33.3%)	5 (31.3%)	3 (27.3%)	1 (16.7%)
None	1 (8.3%)	1 (6.3%)	1 (9.1%)	1 (16.7%)

achieved a slightly higher Area Under the Curve (AUC) of 0.97 (95% CI: 0.91–0.99), while M-CSF achieved an AUC of 0.95 (95% CI: 0.88–0.98).

Sensitivity and Specificity

At optimal cut-offs, VEGF (>182.8 pg/mL) achieved 95% sensitivity (95% CI: 83.1%–99.4%) and 93% specificity (95% CI: 81.3%–98.5%). M-CSF (>883.5 pg/mL) showed 84% sensitivity (95% CI: 70.2%–93.2%) and 100% specificity (95% CI: 92.1%–100%).

Combined Model and Multivariate Analysis

The combined model (Model C) demonstrated superior diagnostic performance with an AUC of 0.974 (95% CI: 0.950–0.998), significantly outperforming M-CSF alone (p = 0.038) (Table 5).

Furthermore, multivariate logistic regression confirmed that both VEGF and M-CSF remained significant independent predictors of cervical cancer after adjusting for age, smoking status, and HPV vaccination status (Table 6).

Association with HPV Vaccination Status

VEGF levels were significantly lower in vaccinated patients (352 ± 120 pg/mL) compared to non-vaccinated patients (428 ± 121 pg/mL, p = 0.047) (Table 7). No significant difference was observed in M-CSF levels (p = 0.74).

Histological Type and Grade

The majority of cases were Squamous Cell Carcinoma (84.4%), with 15.6% being Adenocarcinoma. The most common tumor grade was Grade 2 (48.9%) (Table 8). No significant difference in biomarker levels was found between histological types.

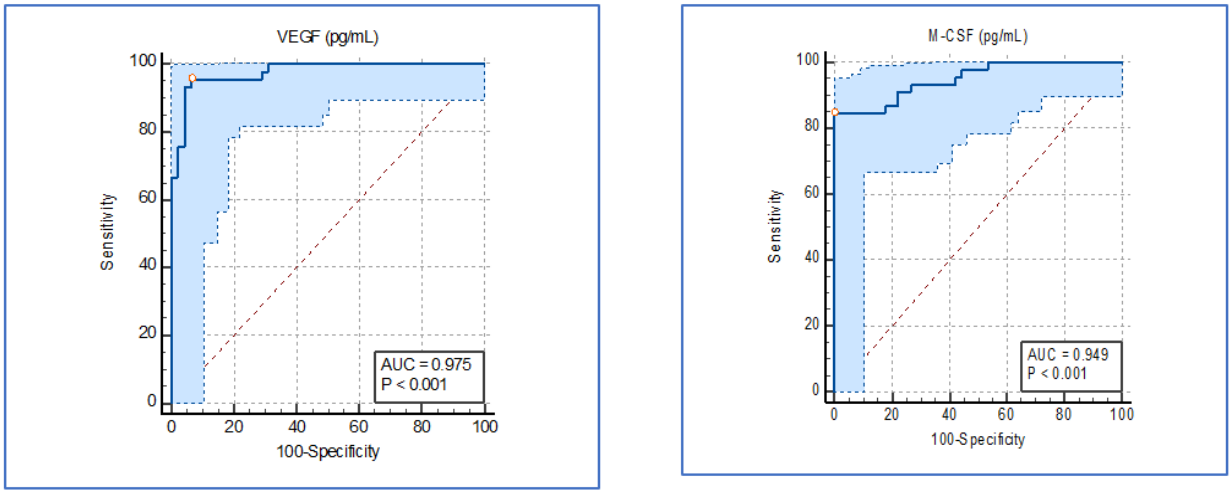

Figure 1. Receiver Operating Characteristic (ROC) Curves Showing the Diagnostic Performance of Serum VEGF and M-CSF in Distinguishing Cervical Cancer Patients from Healthy Controls.

Table 4. Diagnostic Performance of Serum M-CSF and VEGF in Discriminating Cervical Cancer Patients from Healthy Controls

Biomarker	AUC (95% CI)	p-value	Optimal Cut-off Value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Youden's Index (J)
M-CSF	0.95 (0.88 – 0.98)	<0.001	> 883.5	84(70.2%–93.2%)	100(92.1%–100%)	0.84
VEGF	0.97 (0.91 – 0.99)	<0.001	> 182.8	95(83.1%–99.4%)	93(81.3%–98.5%)	0.89

Table 5. Comparison of Diagnostic Performance of Multivariable Logistic Regression Models Using Serum Biomarkers for Cervical Cancer Detection

Model	AUC (95% CI)	p-value‡ (vs. Model A)	AIC	Sensitivity (%)	Specificity (%)
A. M-CSF Only	0.951 (0.914 – 0.988)	(Reference)	98.5	93.3	88.9
B. VEGF Only	0.941 (0.899 – 0.983)	0.421	103.2	91.1	86.7
C. M-CSF + VEGF	0.974 (0.950 – 0.998)	0.038	90.1	95.6	91.1

‡ DeLong's test for two correlated ROC curves; AIC, Akaike Information Criterion (lower is better).

Table 6. Multivariate Logistic Regression Analysis for Cervical Cancer Prediction (n=90)

Variable	Odds Ratio (OR)	95% Confidence Interval	P-value
Serum VEGF (per pg/mL)	1.0162	1.0105 – 1.0220	< 0.001
Serum M-CSF (per pg/mL)	1.0035	1.0020 – 1.0050	< 0.001
Age (per year)	1.02	0.98 – 1.06	0.35
Smoking Status (Smoker vs. Non-Smoker)	1.85	0.75 – 4.56	0.18
HPV Vaccination Status (Vaccinated vs. Non-Vaccinated)	0.55	0.21 – 1.44	0.23

Note: The model adjusts for age, smoking status, and HPV vaccination status. Both VEGF and M-CSF remain significant independent predictors.

Table 7. Association Between HPV Vaccination Status and Serum Biomarker Levels in Cervicaal Cancer Patients

Biomarkers		Vaccinated n = 16	Not Vaccinated n = 29	P. Value
M-CSF (pg/mL)	Mean ±SD	1398 ± 428	1337 ± 590	0.74 [a]
VEGF (pg/mL)	Mean ±SD	352 ± 120	428 ± 121	0.047 [a] *

n, number of cases; SD, standard deviation; ** P <0.00, statistical significance. [a], Independent sample t-test.

Discussion

In this case–control study, serum M-CSF and VEGF were markedly higher in women with cervical cancer than in healthy controls. Both biomarkers rose with advancing FIGO stage, VEGF showed a modest reduction among HPV-vaccinated patients, and each analyte offered excellent single-marker diagnostic discrimination (AUC ≥0.95), with the combined logistic model (M-CSF+VEGF) performing best. These data position circulating M-CSF and VEGF as complementary indicators of tumor biology with potential roles in diagnosis support and disease monitoring, while underscoring that HPV testing remains the primary screening tool.

Elevated circulating VEGF in cervical cancer has been reported in multiple studies. Zusterzeel, Span [23] showed that higher pretreatment serum VEGF independently predicted poorer outcomes in cervical carcinoma, and that levels tended to increase with stage, consistent with our stage-gradient pattern. Marni, Mulawardhana [24] likewise reported correlations between serum VEGF, tumor burden, and clinicopathological parameters, including higher levels in advanced disease. Later datasets e.g.,Winata and Christyani [25] also linked elevated VEGF with worse response and diagnostic utility, although not all studies found unequivocal prognostic value, reflecting cohort and assay heterogeneity.

Table 8. Distribution of Histological Type and Tumor Grade (n=45)

Characteristic	n	%
Histological Type		
Squamous Cell Carcinoma (SCC)	38	84.4
Adenocarcinoma	7	15.6
Tumor Grade		
Grade 1 (Low)	8	17.8
Grade 2 (Moderate)	22	48.9
Grade 3 (High)	15	33.3

Concordant with the findings of the present study, two independent Polish cohorts demonstrated that both VEGF and M-CSF concentrations are significantly higher in cervical cancer than in controls and outperform several traditional markers in ROC analyses; importantly, combining markers further improved accuracy precisely what the present study observed with multivariable model [12, 26]. A focused meta-analysis dedicated to M-CSF estimated pooled sensitivity and specificity of ~70% and ~84% (AUC≈0.86) for differentiating cervical cancer from non-cancer, supporting its value as a blood-based adjunct [13].

While previous studies, such as Sidorkiewicz et al. (2019), have established the diagnostic utility of VEGF and M-CSF, our study provides several novel contributions. Specifically, we offer a detailed analysis of the correlation between these biomarkers and the FIGO 2018 staging system, providing a deeper understanding of their value in assessing disease severity. Furthermore, this is the first study (to our knowledge) to investigate the association between HPV vaccination status and serum VEGF/M-CSF levels in cervical cancer patients, revealing a significant reduction in VEGF levels among vaccinated individuals. Our comprehensive multivariate analysis also strengthens the conclusion that both markers are independent predictors of cervical cancer.

Beyond association studies, The results of present study are biologically coherent with the therapeutic

success of targeting angiogenesis in advanced disease. The GOG-240 randomized trial established that adding bevacizumab (anti-VEGF-A) to chemotherapy improves overall survival, findings confirmed in the final analysis and now embedded in practice [27]. Together, this body of evidence reinforces the clinical relevance of circulating angiogenic signaling in cervical cancer.

VEGF upregulation in cervical cancer is plausibly driven by hypoxia-responsive pathways and direct effects of high-risk HPV oncoproteins [28]. HPV16/18 E6 and E7 increase HIF-1α stability and transcriptional activity, which in turn induces VEGF expression; experimental work demonstrates this axis in cervical cancer cells and tissues [29]. The observed rise of VEGF across stages in the present study is therefore consistent with progressive hypoxia, neovascular remodeling, and angiogenic switch activation.

M-CSF (CSF1) is a central regulator of monocyte–macrophage differentiation and recruitment. Elevated circulating M-CSF may reflect tumor-driven myelopoiesis and the accrual of tumor-associated macrophages (TAMs), which support angiogenesis (via VEGF and other factors), matrix remodeling, and immune suppression. Meta-analytic evidence in cervical cancer links higher TAM density with advanced stage and inferior survival(10). Preclinical studies show that blocking CSF1R reduces TAMs, enhances $CD8^+$ T-cell infiltration, and slows tumor growth including in cervical tumor models highlighting a mechanistic bridge between serum M-CSF levels and an immunosuppressive, pro-angiogenic microenvironment [30, 31].

The lower VEGF observed among vaccinated patients is intriguing. While the present study is not aware of studies directly linking HPV vaccination to systemic angiogenic markers, vaccination is proven to reduce persistent HPV16/18 infection and the incidence of CIN3+ and cervical cancer at a population level. This may be due to the vaccine-induced immune response reducing chronic HPV-driven inflammation and angiogenesis. The mechanism could involve a reduction in chronic viral load, leading to decreased activity of the HIF-1α pathway, which is known to drive VEGF expression, or a shift towards a less pro-tumor immune profile.

Reduced chronic HPV-driven epithelial injury and inflammation could secondarily dampen pro-angiogenic signaling, offering a biologically plausible explanation that warrants prospective validation [5, 6, 32].

Clinical implications

Adjunctive diagnosis/triage where our ROC and Youden's index results suggest that M-CSF and VEGF could assist in distinguishing cancer from benign conditions in symptomatic women or in equivocal imaging scenarios as adjuncts, not as screening tests. Current screening remains HPV-based per WHO guidance; blood biomarkers should not replace HPV testing or colposcopy [33].

Also staging and monitoring where the stage-wise increases imply potential for risk stratification and for tracking treatment response or recurrence, particularly for centers where serial imaging is constrained. Prior studies show serum VEGF falls after effective therapy, consistent with this proposal [27] .

Furthermore, the concordance between high VEGF and benefit from anti-VEGF therapy provides a rationale to explore whether baseline or dynamic VEGF could refine patient selection for anti-angiogenic or combined regimens strictly as a hypothesis-generating avenue pending prospective evidence [27]. Traditional biomarkers like SCC-Ag and CA-125 typically exhibit lower sensitivity (30-50% for SCC-Ag) compared to the markers in our study. VEGF (Sensitivity = 95%) and M-CSF (Sensitivity = 84%) offer significantly superior diagnostic performance, particularly in terms of sensitivity, suggesting they could serve as highly effective adjunct biomarkers, potentially outperforming traditional markers in screening and diagnostic triage settings.

The observation that 16.7% of Stage IV patients were asymptomatic is clinically noteworthy. This may be explained by the incidental discovery of the cancer during routine examinations, the presence of limited metastasis that has not yet caused overt symptoms, or the subjective nature of symptom reporting.

Strengths and limitations

Strengths include the inclusion of well-phenotyped cases and controls; concurrent measurement of two mechanistically linked biomarkers; stage-stratified analyses; and rigorous diagnostic modeling that compared single-marker vs combined performance.

Limitations merit caution. First, because the study only included healthy controls rather than patients with benign gynecologic conditions (e.g., fibroids or endometriosis), the reported diagnostic accuracy may be overestimated compared with real-world clinical scenarios. In clinical practice, distinguishing cervical cancer from other benign conditions may be more challenging. The single-center design and modest sample size limit generalizability and precision of subgroup estimates (e.g., vaccination strata). The current study involves a limited number of participants (n=90), particularly in the subgroups (e.g., Stage IV, n=6). While the current sample provides sufficient statistical power for the primary analysis (cancer vs. control), the subgroup analyses (by stage and vaccination) are considered exploratory and require confirmation in larger, external validation studies. We did not adjust biomarker–outcome relationships for all potential confounders (e.g., exact tumor histology distribution, comorbid inflammation, or HPV genotype load). HPV genotype data was not collected, which limits the ability to explore the influence of specific high-risk types on biomarker expression. Biomarkers were assessed cross-sectionally rather than longitudinally, precluding conclusions about kinetics with treatment or recurrence. Finally, while bleeding and dyspareunia were frequent in patients as expected from guideline summaries symptoms alone are insufficiently specific and should not guide biomarker testing without standard diagnostic pathways [34]. A formal assessment of inter-observer agreement for staging and symptom classification was not performed, as we relied on standardized clinical assessments by gynecologic oncologists.

Future directions

Future work should focus on validating these findings in larger, multi-center cohorts with prespecified cut-offs and independent test sets, as emphasized in recent diagnostic biomarker studies. Longitudinal research is needed to evaluate whether peri-treatment changes in M-CSF and VEGF predict treatment response, progression-free survival, or recurrence, consistent with prior evidence showing serum VEGF declines after effective therapy. Integrated risk models that combine these biomarkers with clinical variables, HPV metrics, and established tumor markers such as squamous cell carcinoma antigen (SCC-Ag) may further enhance discrimination, in line with earlier reports where multi-marker panels outperformed single markers(12). Finally, mechanistic and vaccination-focused studies should explore how the CSF1–VEGF–TAM axis influences circulating biomarkers and whether HPV vaccination modifies systemic angiogenic or inflammatory profiles in cervical cancer, reflecting prior work linking TAM density to prognosis and HPV vaccination to reduced disease incidence (31).

In conclusion, serum VEGF and M-CSF demonstrate excellent diagnostic utility for cervical cancer, with VEGF alone achieving an AUC of 0.97 (95% CI: 0.91–0.99) with 95% sensitivity and 93% specificity, and M-CSF achieving an AUC of 0.95 (95% CI: 0.88–0.98) with 84% sensitivity and 100% specificity. They also showed a strong association with disease stage ($p = 0.0073$ and $p = 0.0006$, respectively). The combined model (AUC 0.974) significantly enhances discrimination compared to M-CSF alone ($p = 0.038$). These findings support their potential as adjunct biomarkers in diagnosis and clinical assessment, but external validation and longitudinal studies are required to confirm these results in diverse clinical contexts

Author Contribution Statement

Ahmed Jasim Mohammed: Conceptualization, methodology, draft preparation. Suhad Abdulhussein Salman: Data collection, analysis, manuscript editing. Abbas Chyad: Statistical analysis, literature review. Redha Dawud Abd Alredha: Supervision, final manuscript revision, corresponding author.

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Institutional Approval

The study was approved by Al-Qadisiyah General Hospital, Al-Diwaniyah Governorate, Iraq. This study is part of independent research and was not part of an approved student thesis.

Conflicts of Interest/Competing Interests

The authors declare no conflicts of interest.

Ethics Approval

The study was approved by the Ethics Committee of Al-Qadisiyah General Hospital, Al-Diwaniyah Governorate, Iraq (Approval No. M240906, dated September 2, 2024).

Availability of Data and Materials

Data are available from the corresponding author upon reasonable request.

Study Registration

This study was not registered in any registration dataset (such as ClinicalTrials.gov) as it is a diagnostic case-control study and not a clinical trial or systematic review.

Inter-Observer Agreement

Disease stage (FIGO 2018) was determined by gynecologic oncologists according to standardized criteria. Symptom classification was recorded via a structured form. A formal assessment of inter-observer agreement for staging or symptom classification was not performed, as we relied on standardized clinical assessments.

List of Abbreviations

HPV: human papillomavirus
WHO: World Health Organization
VEGF: vascular endothelial growth factor
M-CSF: macrophage colony-stimulating factor
TAM(s): tumor-associated macrophage(s)
CSF1R: colony-stimulating factor 1 receptor
ROC: receiver operating characteristic
AUC: area under the curve
CI: confidence interval

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