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Original Article

Development of a prognostic model for cervical cancer based on inflammation-related gene signatures and immune microenvironment regulation

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Abstract: Aims: To develop a prognostic model for cervical cancer by integrating inflammation-related gene signatures and clinical factors, aimed at improving patient outcome prediction and exploring the role of immune microenvironment regulation in tumor progression. Methods: This retrospective study analyzed 150 cervical cancer patients from Taiyuan Maternal and Child Health Hospital, diagnosed between January 2019 and January 2022. Patients were categorized into a poor prognosis (n=60) and a good prognosis group (n=90) based on prognosis. The study evaluated the prognostic value of inflammation-related gene expression on overall survival (OS) and progression-free survival (PFS). MMP-9, IL-6, and COX-2 expression levels were stratified as high or low based on median values. Results: Pro-inflammatory genes (TNF-alpha, IL-6, MMP-9, and COX-2) were significantly higher in the poor prognosis group, while IL-10 levels were lower (all $P<0.001$). Multivariate analysis identified independent risk factors for poor prognosis, including HPV infection status, MMP-9, IL-6, TNF-alpha, and lymph node involvement. A nomogram incorporating these factors demonstrated strong discrimination (AUC 0.83) and effective poor outcome prediction. Elevated levels of MMP-9, IL-6, and COX-2 correlated with poor OS and PFS, highlighting their potential as prognostic markers and therapeutic targets. These gene expression alterations were also associated with immune microenvironment dysregulation, suggesting their role in immune evasion and chronic inflammation in tumors. Conclusion: A prognostic model for cervical cancer was developed integrating inflammation-related gene signatures and clinical factors. Elevated MMP-9, IL-6, and COX-2 expression are linked to poor prognosis and immune microenvironment disruption, offering a promising approach for personalized treatment.

Keywords: Cervical cancer, prognostic model, inflammation-related genes, matrix metalloproteinase-9, interleukin 6, immune microenvironment

Introduction

Cervical cancer is a leading cause of cancer-related deaths among women worldwide, particularly in regions with limited resources [1-3]. A significant number of patients are diagnosed at advanced stages, which limits the effectiveness of available treatments and increases the risk of death [4-6]. The complexity of cervical cancer prognosis is attributed to the influence of genetic, environmental, and immune system factors [7-10]. Although traditional prognostic systems are practical, they fail to capture the full spectrum of underlying tumor changes, highlighting the need for more precise patient classification markers.

The progression of cervical cancer is largely influenced by the tumor microenvironment (TME), which consists of tumor cells, stromal cells, immune cells, the extracellular matrix, and various cytokines and growth factors [11, 12]. Inflammation within the TME promotes tumor development, facilitates metastasis, and aids immune evasion. Gene signatures related to inflammation, including cytokines, matrix metalloproteinases (MMPs), and immune checkpoint regulators, are crucial in shaping both tumor behavior and the immune response [13]. These molecules not only contribute to cancer progression but also influence how the body responds to treatment.

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Advances in molecular biology have provided insights into the role of inflammation and immune responses in cervical cancer [14]. However, these molecular signatures have yet to be fully integrated into clinical practice. While some biomarkers can predict prognosis, a comprehensive model that combines both inflammation-related and immune factors is still lacking. Inflammation-related gene signatures refer to a set of genes whose collective expression reflects the extent of inflammatory activation in the TME. These signatures typically include cytokines (e.g., IL-6 and TNF-alpha), chemokines, enzymes involved in prostaglandin production (e.g., COX-2), connexins, and matrix-remodeling proteins (e.g., MMP-9) [15]. Together, these genes play pivotal roles in regulating immune cell recruitment, angiogenesis, extracellular matrix degradation, and tumor immune evasion. Their expression provides insights into the inflammatory state of cervical cancer tissues and may serve as promising indicators for understanding disease progression and patient prognosis.

This study aims to bridge the gap by developing a prognostic model for cervical cancer that incorporates both inflammation-related gene data and tumor response factors, including clinical and immune responses. The model seeks to predict disease progression and identify patients who may benefit from targeted therapies based on the evaluation of a cluster of inflammation-related genes. The goal is to pinpoint the key determinants of cervical cancer outcomes to inform personalized treatment strategies, enhancing the likelihood of successful interventions.

Materials and methods

Case selection

This retrospective study analyzed 150 patients diagnosed with cervical cancer at our institution between January 2019 and January 2022. The cohort was divided into two groups based on prognosis: the poor prognosis group (n=60) and the good prognosis group (n=90). Patients in the poor prognosis group were characterized by [16]: (1) disease progression, indicated by the presence of metastasis or an increase in the size of the primary tumor despite treatment, (2) relapse, defined as cancer recurrence after a symptom-free period following initial treatment [17], and (3) mortality, where death was attributed to cervical cancer or its complications during the follow-up period [18].

The good prognosis group consisted of patients who survived the follow-up period without progression or recurrence, with stable disease characterized by tumor-free progression and maintained quality of life.

The cohort was further divided into a training set (n=90) and a validation set (n=60). The prognostic model was developed using the training set, and its performance and predictive accuracy were evaluated using the validation set. Inclusion criteria were: (1) histologically confirmed cervical cancer diagnosis, (2) complete clinical and follow-up data, and (3) at least three years of follow-up.

Exclusion criteria included: (1) incomplete clinical or follow-up data, (2) secondary malignancies or severe systemic diseases (e.g., cardiovascular, renal, or liver diseases) affecting prognosis or treatment outcomes, (3) non-standard cancer therapies (e.g., experimental treatments), (4) insufficient follow-up data (less than three years), (5) significant comorbidities (e.g., autoimmune diseases, uncontrolled diabetes), (6) histologically confirmed non-epithelial cervical tumors, and (7) other malignancies within five years, excluding non-melanoma skin cancer. The flowchart of the experimental design was shown in the **Figure 1**. Ethical approval was granted by the Ethics Committee of Taiyuan Maternal and Child Health Hospital, and all patient management adhered to the ethical guidelines of the Declaration of Helsinki.

Data collection

Clinical data, including patient age, tumor type, stage, histological grade, HPV status, lymph node involvement, and tumor size, were assessed. Tumor staging followed the International Federation of Gynecology and Obstetrics system, and histological grading was based on the World Health Organization classification. HPV presence was detected by PCR, primarily targeting HPV types 16 and 18. Tumor measurements and staging were determined using imaging technologies, and lymph node involvement was confirmed through clinical examination and radiological tools.

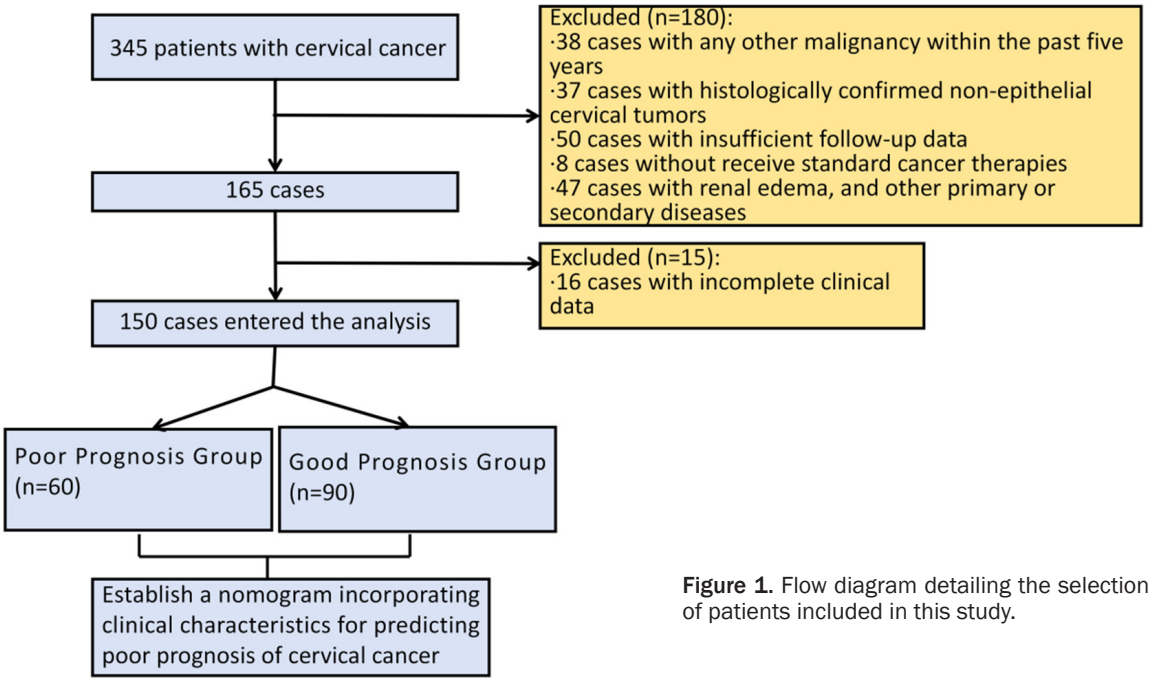

Figure 1. Flow diagram detailing the selection of patients included in this study.

Peripheral blood samples were collected from all patients at diagnosis. Peripheral blood mononuclear cells were isolated using Ficoll-Paque (GE Healthcare Life Sciences) density gradient centrifugation. Approximately 5-10 mL of whole blood was diluted with an equal volume of PBS, layered onto Ficoll-Paque solution, and centrifuged at 400 × g for 30 minutes. PBMCs were collected from the interface, washed with PBS, and stored in RNA stabilizing solution.

RNA extraction was performed using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. RNA quality was assessed using a NanoDrop 2000 spectrophotometer to measure the A260/A280 ratio, with samples having a ratio above 1.8 used for further analysis. cDNA synthesis was carried out using the PrimeScript RT Reagent Kit (Takara, Japan) from 1 μg of total RNA. Reverse transcription was conducted at 37°C for 15 minutes, followed by 85°C for 5 seconds in a 20 μL reaction volume.

Quantitative PCR (qPCR) was performed using SYBR Green PCR Master Mix (Applied Biosystems, USA) on an ABI 7500 Real-Time PCR System. The amplification program included initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

The relative expression of target genes was determined using the 2^-ΔΔCt method, where ΔCt was the difference between the Ct value of the target gene and the Ct value of GAPDH, and ΔΔCt represented the difference between the experimental and control groups (**Table 1**).

Outcome measurement

The primary outcome measures in this study were overall survival (OS) and progression-free survival (PFS), which were used to assess the prognostic significance of inflammation-related gene expression in cervical cancer patients. OS was defined as the time from diagnosis to death from any cause, while PFS was measured from diagnosis to the first occurrence of disease progression or relapse [19]. Clinical outcome data were obtained from patients' medical records, with follow-up conducted at regular intervals after treatment initiation. To evaluate the prognostic value of inflammation-related biomarkers, patients were stratified into high and low expression groups based on the median expression levels of key genes, including MMP-9, IL-6, and COX-2.

Sample size calculation

Sample size calculation was performed using G*Power software (version 3.1). The primary objective was to detect significant differences

Table 1. The following primers were used for gene amplification

Gene	Forward Primer	Reverse Primer
TNF-α	5'-GAGGCAACCTGACCCTGA-3'	5'-AGTGACAGGCAGTGAGTGGA-3'
IL-6	5'-GAGGAGACTTGCAGAAAACC-3'	5'-AGGTTTCCGCCATGGAGG-3'
IL-1β	5'-GGAGAAAGAGGACCAAGG-3'	5'-GGGTTAGAGGAGTAGGG-3'
NF-κB	5'-CAGCAGGAAGATGGAGG-3'	5'-GAGAGGAGAGTAGAGGAAG-3'
CXCL8 (IL-8)	5'-GTTAGGAGAGGATGAGGAG-3'	5'-CTGAAGGAGGAGAGGGAG-3'
IL-10	5'-CTGCAGAGGTTTGAGGAG-3'	5'-CAGGGAGAGGAAGGAGAG-3'
COX-2	5'-CCGGAGAGGGAGGAAGGG-3'	5'-GAGGAGGAGGAGGAAGGG-3'
MMP-9	5'-GCTGAGTGAGAGTAGAAAG-3'	5'-GAGAGGAGGAAGGAGGG-3'
TGF-β1	5'-TGGAGAGCAGGAAGGAG-3'	5'-GAGAGGAGGAGGAAGGG-3'
CRP	5'-ACACAGTTGGAAAGTGAGG-3'	5'-GCTCTGAAGTGGCGGCG-3'
GAPDH	5'-GAGGCAGGATCCCTCCAAAT-3'	5'-GGTGTTGTGCTATCTCTCATGG-3'

in the expression levels of inflammation-related genes between patients with poor and good prognosis, and to develop a robust prognostic model with sufficient statistical power. Based on prior studies reporting effect sizes (Cohen's d) between 0.5 and 0.7 for gene expression differences in similar cancer populations, we assumed a moderate effect size of 0.6. To achieve a statistical power (1-β) of 0.80 and a significance level (α) of 0.05 for a two-tailed test comparing two independent groups, the minimum required sample size per group was estimated to be 45 patients. Given that multivariate logistic regression analyses would include up to 8 variables, we applied the rule of thumb of at least 10 events per variable to ensure model stability and avoid overfitting. Assuming an event rate of approximately 40% (patients experiencing poor prognosis), a total sample size of at least 200 patients would be required. Due to patient availability constraints during the study period, the final cohort included 150 patients (60 with poor prognosis and 90 with good prognosis). While this sample size meets the minimum requirement for detecting gene expression differences and developing preliminary prognostic models, expanding the cohort in future studies would enhance statistical power, enable external validation, and improve the generalizability of the findings.

Statistical methods

Data analyses were conducted using SPSS version 22.0 and R software, version 4.0.3. For normally distributed data, means ± SD were reported, and comparisons between groups were made using the t-test. For skewed data, median values were reported and compared using the Mann-Whitney U test. Categorical variables were presented as counts and percentages, with the Chi-square test test applied when appropriate. Kaplan-Meier curves were used to assess the value of gene signatures in predicting OS and PFS, with differences between groups tested using the log-rank test. Univariate and multivariate Cox proportional hazards regression models were used to identify independent factors predicting OS and PFS. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for each factor, with a *P*-value <0.05 considered statistically significant.

To predict the risk of poor prognosis in cervical cancer, a nomogram was developed based on significant clinical and molecular factors identified in regression analysis. The rms package in R was used to build the nomogram, assigning points based on regression coefficients. The final score was derived by summing the points, representing the likelihood of a poor prognosis. Calibration curves were plotted to assess whether the predicted values aligned with the actual outcomes. The AUC for the receiver operating characteristic (ROC) curve was calculated for the nomogram, with an AUC greater than 0.7 indicating good predictive accuracy. The clinical utility of the nomogram was further evaluated using Decision Curve Analysis (DCA), which assessed whether the model provided better clinical value than simple risk-based or no intervention strategies. All statistical tests were two-sided, and *P*-values <0.05 were considered significant.

Table 2. Comparison of clinical characteristics between the poor and good prognosis groups

Parameter	Poor Prognosis Group (n=60)	Good Prognosis Group (n=90)	*P*-value
Age (years)	54.78±9.62	48.88±8.49	<0.001
Tumor Stage			<0.001
Stage I	8 (13.3%)	32 (35.6%)	
Stage II	12 (20.0%)	30 (33.3%)	
Stage III	28 (46.7%)	18 (20.0%)	
Stage IV	12 (20.0%)	10 (11.1%)	
Histological Grade			<0.001
Well-differentiated	6 (10.0%)	34 (37.8%)	
Moderately differentiated	18 (30.0%)	38 (42.2%)	
Poorly differentiated	36 (60.0%)	18 (20.0%)	
HPV Infection Status			0.041
HPV 16/18 positive	42 (70.0%)	48 (53.3%)	
HPV negative	18 (30.0%)	42 (46.7%)	
Lymph Node Involvement			<0.001
Positive	48 (80.0%)	28 (31.1%)	
Negative	12 (20.0%)	62 (68.9%)	
Treatment Modality			<0.001
Radical Surgery	12 (20.0%)	70 (77.8%)	
Chemotherapy	24 (40.0%)	32 (35.6%)	
Radiation Therapy	18 (30.0%)	30 (33.3%)	
Palliative Care	6 (10.0%)	0 (0.0%)	
Tumor Size (cm)	4.55±1.00	3.26±0.89	<0.001

Note: HPV: Human Papillomavirus.

Results

Comparison of clinical characteristics between the poor and good prognosis groups

Comparison of clinical characteristics between the groups revealed significant differences across several variables. Patients in the poor prognosis group were older (54.78±9.62 vs. 48.88±8.49 years, P<0.001) and more likely to present with advanced tumor stages (Stage III-IV: 66.7% vs. 31.1%, P<0.001). Poorly differentiated tumors were more common in this group (60.0% vs. 20.0%, P<0.001), whereas well-differentiated tumors were more common in the good prognosis group. HPV 16/18 positivity was higher in the poor prognosis group (70.0% vs. 53.3%, P=0.041). Additionally, lymph node involvement was more frequent (80.0% vs. 31.1%, P<0.001), and the average tumor size was larger (4.55±1.00 cm vs. 3.26±0.89 cm, P<0.001). Treatment patterns also differed: radical surgery was more frequently performed in the good prognosis group (77.8% vs. 20.0%, P<0.001), while patients in the poor prognosis group had higher rates of chemotherapy and palliative care (**Table 2**).

Comparison of the relative mRNA expression levels of inflammation-related genes between the poor and good prognosis groups

As shown in **Figure 2**, the poor prognosis group exhibited significantly higher expression levels of pro-inflammatory genes (P<0.001). TNF-α (**Figure 2A**) levels were notably higher in the poor prognosis group compared to the good prognosis group (P<0.001). Similarly, IL-6 levels were significantly higher in the poor prognosis group (P< 0.001) (**Figure 2B**), indicating an increased inflammatory response linked to poor clinical outcomes. In addition to TNF-α and IL-6, other pro-inflammatory markers, such as IL-18, NF-κB, and CXCL8 (**Figure 2E**), were significantly upregulated in the poor prognosis group (all P<0.001). IL-1β expression was higher in the poor prognosis group compared to the good prognosis group (P<0.05) (**Figure 2C**), and NF-κB levels were also significantly elevated in the poor prognosis group (P<0.01) (**Figure 2D**). Moreover, COX-2, MMP-9, and CRP (**Figure 2G-J**) expression levels were significantly higher in the poor prognosis group (all P<0.001), suggesting their involvement in tumor progression and inflammatory pathways. In contrast, the anti-inflammatory cytokine IL-10 showed lower expression in the poor prognosis group (P<0.05) (**Figure 2F**), reflecting an imbalance in the inflammatory response.

Multivariate regression analysis of independent risk factors for poor prognosis of cervical cancer

Multivariate logistic regression analysis identified several significant independent risk factors associated with poor prognosis in cervical can-

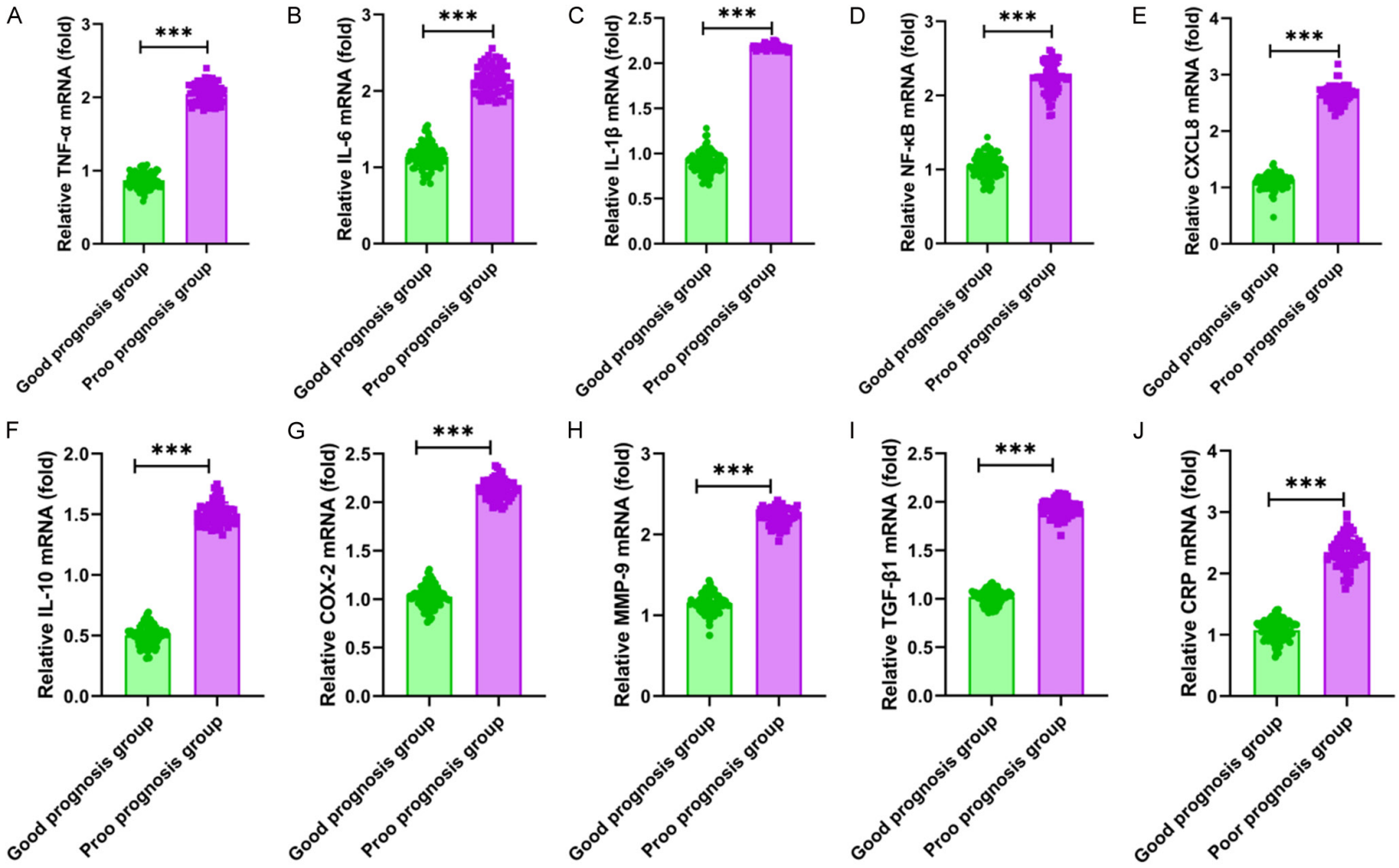

Figure 2. Comparison of the relative mRNA expression levels of inflammation-related genes between two groups. (A) TNF-α, (B) IL-6, (C) IL-1β, (D) NF-KB, (E) CXCL8, (F) IL-10, (G) COX-2, (H) MMP-9, (I) TGF-β1, (J) CRP. Compare to the good prognosis group, ***P<0.001. Note: TNF-α: Tumor Necrosis Factor Alpha; IL-6: Interleukin 6; IL-1β: Interleukin 1 Beta; NF-κB: Nuclear Factor Kappa B; CXCL8: C-X-C Motif Chemokine Ligand 8; IL-10: Interleukin 10; COX-2: Cyclooxygenase 2; MMP-9: Matrix Metalloproteinase 9; TGF-β1: Transforming Growth Factor Beta 1; CRP: C-Reactive Protein.

Table 3. Multivariate regression analysis of independent risk factors for poor prognosis of cervical cancer

Variable	B	SE	Wald	P	OR	95% CI
HPV Infection Status	2.081	0.506	16.914	<0.001	0.125	0.046-0.336
MMP-9 (>2.23)	0.080	0.030	7.084	0.008	0.923	0.870-0.979
IL-6 (>2.10)	1.338	0.452	8.777	0.003	0.262	0.108-0.636
COX-2 (>2.12)	1.033	0.463	4.981	0.026	2.808	1.134-6.955
Lymph Node Involvement	0.381	0.074	26.206	<0.001	1.464	1.265-1.694
TNF-α (>2.00)	0.709	0.154	21.250	<0.001	2.032	1.503-2.746
Constant	11.950	3.444	12.038	0.001	0.000	-

Note: HPV: Human Papillomavirus; MMP-9: Matrix Metalloproteinase 9; IL-6: Interleukin 6; COX-2: Cyclooxygenase 2; TNF-α: Tumor Necrosis Factor Alpha.

cer. The multivariate model included the following variables: HPV infection status, MMP-9, IL-6, COX-2, TNF-α, and lymph node involvement. These factors were selected based on two criteria: their statistical significance in univariate analyses (all P<0.05), indicating a clear association with clinical outcomes, and their relevance in previous studies, which have demonstrated their roles in inflammation-driven tumor progression, immune suppression, and metastasis. Specifically, HPV infection status (OR: 0.125, 95% CI: 0.046-0.336, P<0.001) was identified as a strong protective factor, indicating that HPV 16/18-positive patients had a significantly lower likelihood of poor prognosis. Elevated MMP-9 levels (>2.23) were associated with favorable outcomes (OR: 0.923, 95% CI: 0.870-0.979, P=0.008). In contrast, increased IL-6 (>2.10, OR: 0.222, 95% CI: 0.108-0.636, P=0.003) showed a protective association as well. However, high expression of COX-2 (>2.12) significantly increased the risk of poor prognosis (OR: 2.808, 95% CI: 1.134-6.955, P=0.026). Lymph node involvement remained a strong independent predictor (OR: 1.464, 95% CI: 1.265-1.694, P<0.001), and elevated TNF-α levels (>2.00) were significantly associated with poor outcomes (OR: 2.032, 95% CI: 1.503-2.746, P<0.001) (**Table 3**).

Clinical characteristics of the training and validation sets

The clinical characteristics of the training set (n=90) and validation set (n=60) were compared to assess their comparability. The average age was 48.91±7.14 years in the training set and 48.40±8.03 years in the validation set, with no significant difference (P=0.683). Tumor stage distribution was similar between the two sets: Stage I (13.3% vs. 16.7%), Stage II (20.0% vs. 16.7%), Stage III (46.7% vs. 50.0%), and Stage IV (20.0% vs. 16.7%), with no statistically significant difference (P= 0.853). Histological grade distribution was also similar: well-differentiated tumors (37.8% vs. 36.7%, moderately differentiated tumors (42.2% vs. 41.7%%), and poorly differentiated tumors (20.0% vs. 21.7%), with no significant difference (P=0.969). HPV 16/18 positivity was present in 53.3% of the training set and 50.0% of the validation set (P=0.689). Lymph node involvement was observed in 31.1% of the training set and 33.3% of the validation set, with no significant difference (P=0.775). Treatment modalities, including radical surgery (77.8% vs. 76.7%), chemotherapy (35.6% vs. 33.3%), radiation therapy (33.3% vs. 35.0%), and palliative care (42.2% vs. 41.7%) were similarly distributed, with no significant differences (P=0.994). Tumor size was 3.71±0.88 cm in the training set and 3.72±0.74 cm in the validation set, with no significant difference (P=0.968) (**Table 4**).

Development of nomogram model

The nomogram for predicting poor outcomes in cervical cancer was developed by combining several important biomarkers and clinical factors. The model incorporates markers such as HPV status, MMP-9 expression, IL-6 levels, COX-2 expression, lymph node involvement, and TNF-α levels. Each risk factor was assigned a threshold value to determine the points, with higher points indicating a higher risk of poor outcomes. The nomogram assigns points based on the following criteria: HPV positivity (positive vs. negative), MMP-9 levels (<2.3 vs.

Table 4. The clinical baseline characteristics of the training and validation sets

Parameter	Training Set (n=90)	Validation Set (n=60)	*P*-value
Age (years)	48.91±7.14	48.40±8.03	0.683
Tumor Stage			0.853
Stage I	12 (13.3%)	10 (16.7%)	
Stage II	18 (20.0%)	10 (16.7%)	
Stage III	42 (46.7%)	30 (50.0%)	
Stage IV	18 (20.0%)	10 (16.7%)	
Histological Grade			0.969
Well-differentiated	34 (37.8%)	22 (36.7%)	
Moderately differentiated	38 (42.2%)	25 (41.7%)	
Poorly differentiated	18 (20.0%)	13 (21.7%)	
HPV Infection Status			0.689
HPV 16/18 positive	48 (53.3%)	30 (50.0%)	
HPV negative	42 (46.7%)	30 (50.0%)	
Lymph Node Involvement			0.775
Positive	28 (31.1%)	20 (33.3%)	
Negative	62 (68.9%)	40 (66.7%)	
Treatment Modality			0.994
Radical Surgery	70 (77.8%)	46 (76.7%)	
Chemotherapy	32 (35.6%)	20 (33.3%)	
Radiation Therapy	30 (33.3%)	21 (35%)	
Palliative Care	38 (42.2%)	25 (41.7%)	
Tumor Size (cm)	3.71±0.88	3.72±0.74	0.968

Note: HPV: Human Papillomavirus.

≥2.3), IL-6 levels (<10 vs. ≥10), COX-2 expression (<12 vs. ≥12), lymph node involvement (positive vs. negative), and TNF-α levels (<20 vs. ≥20) (**Figure 3**). The cumulative points are mapped to a score indicating the likelihood of a poor outcome, with a high risk (0.6 to 0.8) or a low risk (0.1 to 0.2). This nomogram aids in personalizing risk assessment for cervical cancer patients.

Validation of the nomogram model

The nomogram model was validated using calibration curves and ROC curves. As shown in **Figure 4A**, the calibration curve demonstrates moderate agreement between the predicted probabilities from the nomogram and the actual observed outcomes. While the predictions closely match the ideal reference line in the lower and middle probability ranges, some overestimation is observed in the higher probability range (>0.6), as indicated by deviation from the ideal curve. This suggests that, although the model has overall predictive utility, its performance in predicting very high-risk cases is limited and may require further refinement or recalibration in larger, independent cohorts.

Additionally, the ROC curve in **Figure 4B** evaluates the model's ability to distinguish between patient outcomes. With an AUC (Area Under the Curve) of 0.83, the model effectively differentiates between patients with poor and good prognosis. The high AUC indicates that the nomogram is successful in identifying high- and low-risk patients, providing significant predictive power in clinical settings. These findings validate that the nomogram accurately predicts the likelihood of poor outcomes in cervical cancer patients.

DCA

To assess the clinical utility of the nomogram, DCA was conducted, as shown in **Figure 4C**. DCA was used to evaluate the net benefit of applying the nomogram across various probabilities of predicting poor prognosis. The curve labeled "Poor prognosis prediction nomogram" shows a substantial net benefit, particularly at intermediate threshold probabilities (ranging from 0.1 to 0.7). This indicates that the nomogram has significant clinical value in scenarios where risk classification thresholds lie within this range. In comparison, the "All" and "None" curves, which represent scenarios where all or no patients are classified as high-risk, show minimal net benefit. The DCA results emphasize that the nomogram outperforms both extreme strategies, supporting its utility in guiding clinical decision-making and risk stratification for cervical cancer prognosis.

Prognostic analysis of MMP-9, IL-6, and COX-2 expression levels in cervical cancer patients

The expression levels of MMP-9, IL-6, and COX-2 were evaluated as potential prognostic biomarkers in cervical cancer patients. As shown in **Figure 5**, significant differences in

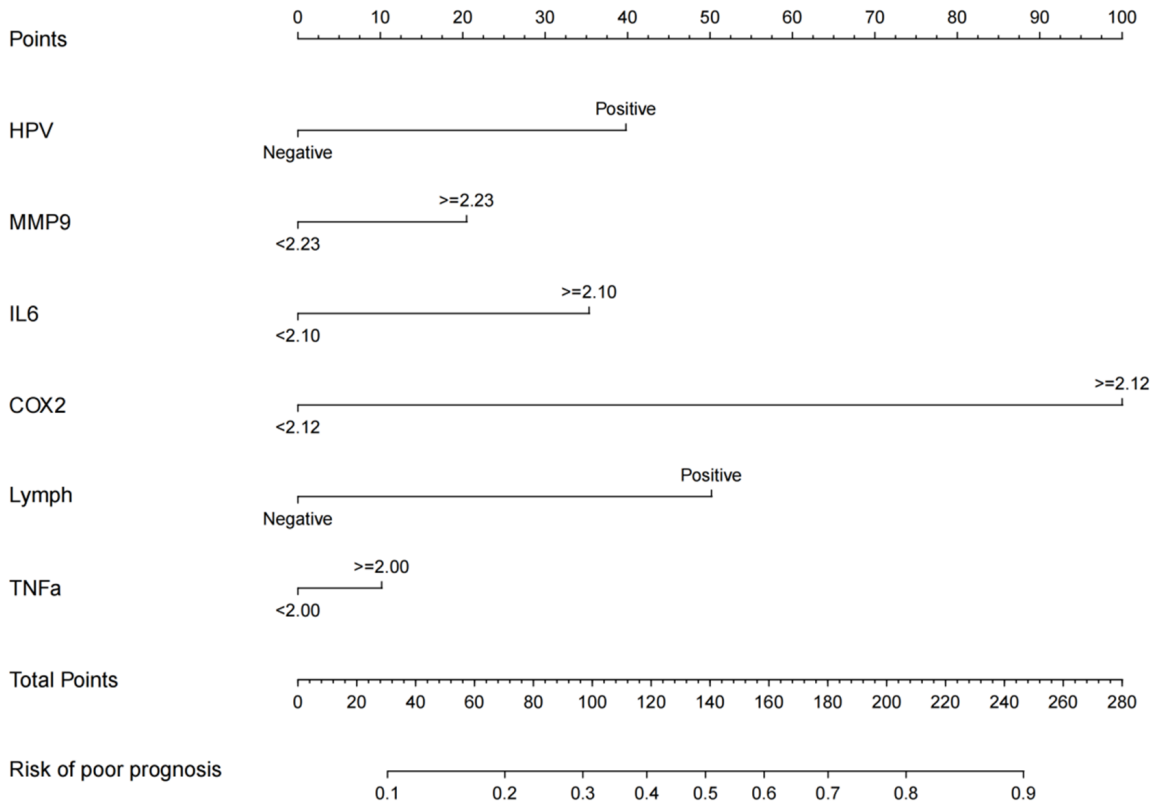

Figure 3. The nomogram for predicting the risk of poor prognosis of cervical cancer. Note: HPV: Human Papillomavirus; MMP9: Matrix Metalloproteinase 9; IL6: Interleukin 6; COX2: Cyclooxygenase 2; TNFα: Tumor Necrosis Factor Alpha.

overall survival and progression-free survival were observed based on the expression levels of these markers. High MMP-9 expression was associated with significantly worse overall survival (P<0.01, **Figure 5A**) and progression-free survival (P<0.01, **Figure 5B**), indicating that MMP-9 plays a crucial role in tumor aggressiveness and metastasis. Similarly, elevated IL-6 levels were correlated with poorer overall survival (P<0.001, **Figure 5C**) and progression-free survival (P<0.001, **Figure 5D**), suggesting that IL-6 contributes to tumor progression and immune evasion in cervical cancer. Lastly, high COX-2 expression was associated with significantly reduced overall survival (P<0.01, **Figure 5E**) and progression-free survival (P<0.01, **Figure 5F**), highlighting its role in promoting tumor growth and metastasis.

Discussion

The new model presented here uses inflammation-related genes to predict the outcome of cervical cancer and explores the relationship between the cancer and the immune system. By focusing on MMP-9, IL-6, and COX-2, we identified key markers that can predict how cervical cancer patients will respond to treatment. When combined with HPV types and lymph node involvement, these markers help create a tool for assessing the risk of cervical cancer.

It is well-established that inflammation plays a critical role in the initiation and progression of cancer. Our research further supports this understanding. The poor prognosis group exhibited significantly higher expression levels of TNF-α, IL-6, and other pro-inflammatory cytokines compared to the good prognosis group. As previous studies have indicated, persistent inflammation in the tumor microenvironment contributes to tumor growth, immune evasion, and metastasis [20, 21]. Elevated levels of IL-6 and TNF-α are consistently associated with worse clinical outcomes in various cancers, including cervical cancer. The high expression of these markers in patients with poor prognosis suggests that chronic inflammation pro-

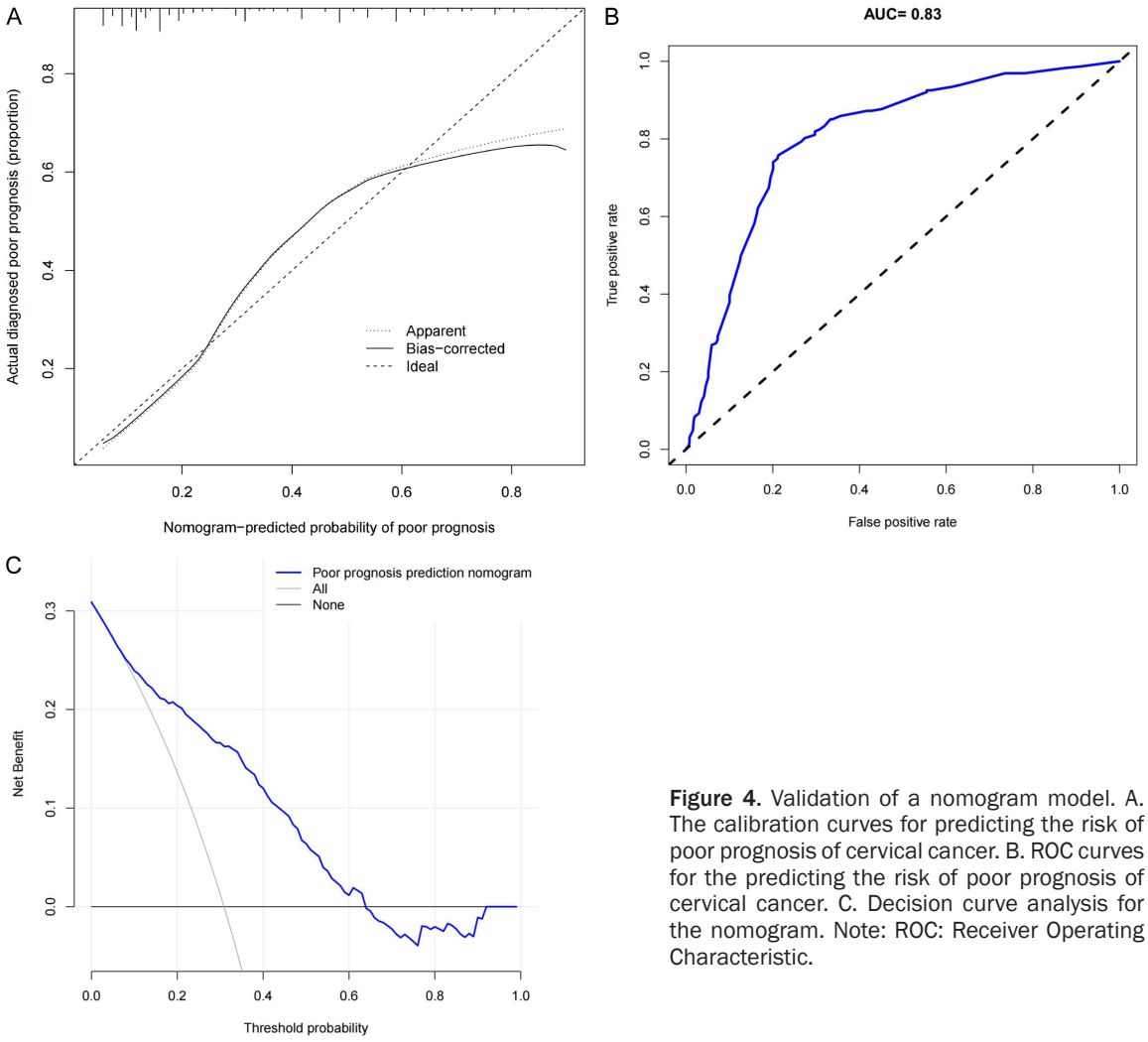

Figure 4. Validation of a nomogram model. A. The calibration curves for predicting the risk of poor prognosis of cervical cancer. B. ROC curves for the predicting the risk of poor prognosis of cervical cancer. C. Decision curve analysis for the nomogram. Note: ROC: Receiver Operating Characteristic.

motes tumor growth and resistance to therapy, highlighting the need for anti-inflammatory strategies in these cases.

We also observed significantly higher expression of MMP-9, COX-2, and CXCL8 in the poor prognosis group, confirming the critical role of extracellular matrix remodeling and immune cell accumulation in cervical cancer progression. These findings align with previous research, which links MMP-9 to cancer metastasis and COX-2 to increased angiogenesis and immune suppression in cervical cancer [22]. Both MMP-9 and COX-2 play crucial roles in the degradation of the extracellular matrix, enabling tumor cells to invade and spread [23]. Targeting these inflammatory markers may prevent cancer metastasis and improve patient recovery. Additionally, elevated CXCL8 levels attract immune cells, potentially creating a TME that suppresses immune activity and supports tumor survival.

Interestingly, in the poor prognosis group, we observed lower levels of the anti-inflammatory cytokine IL-10. When pro-inflammatory cytokines outweigh anti-inflammatory cytokines, an uncontrolled immune response appears to accelerate malignant transformation in cervical cancer cells. Previous studies have shown that IL-10 serves to dampen the immune response, and its reduced expression in the poor prognosis group may hinder effective immune responses against the tumor [24]. Thus, low IL-10 levels likely contribute to unchecked inflammation, allowing the tumor to grow and evade immune detection [25]. This research emphasizes the importance of main-

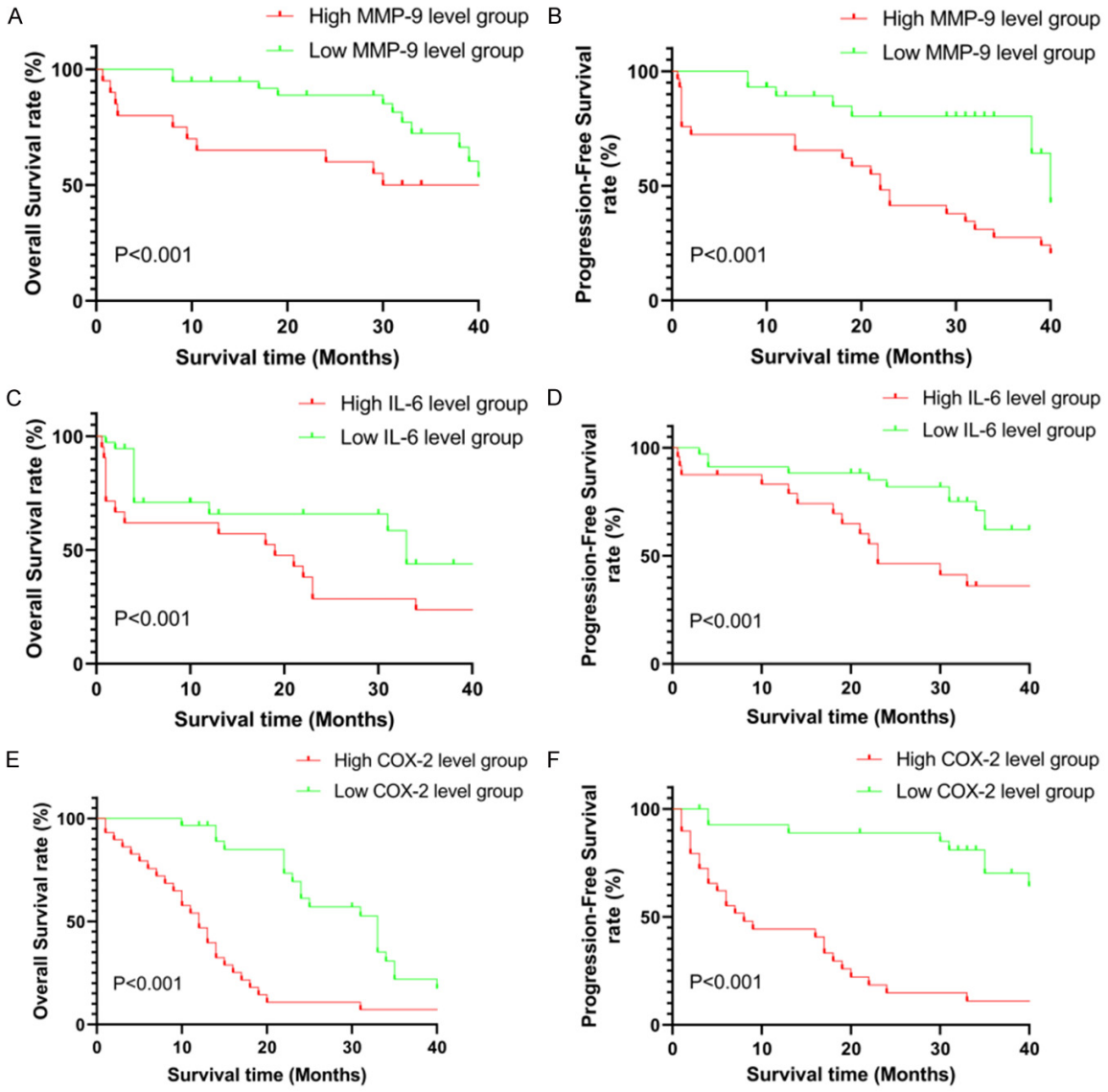

Figure 5. Prognostic analysis of MMP-9, IL-6, and COX-2 expression level in cervical cancer patients. (A) The overall survival rate of different MMP-9 levels, (B) The progression-free survival rate of different MMP-9 levels, (C) The overall survival rate of different IL-6 levels, (D) The progression-free survival rate of different IL-6 levels, (E) The overall survival rate of different COX-2 levels, (F) The progression-free survival rate of different COX-2 levels. Note: MMP-9: Matrix Metalloproteinase 9; IL-6: Interleukin 6; and COX-2: Cyclooxygenase 2.

taining a balanced immune system for better clinical outcomes, suggesting that therapies aimed at rebalancing the immune system are crucial.

Lymph node involvement was also linked to poor prognosis in our study, consistent with previous findings [26]. Cancer involvement of lymph nodes indicates advanced disease and a higher likelihood of recurrence. Increased TNF-α levels in lymph node-positive patients may reflect an immune response triggered by metastasis, with the tumor recruiting immune cells to support its growth [27]. Early detection of lymph node involvement and prompt intervention are essential to prevent cancer spread and improve survival rates.

The TME surrounding immune cells plays a significant role in cervical cancer, primarily through inflammation, which not only promotes the disease but also facilitates immune evasion. Among the main pro-inflammatory cytokines, TNF-α, IL-6 [28], and COX-2 are linked to

cancer development, metastasis, and reduced immune response against the tumor. Reducing inflammation in these pathways could modulate the immune system and improve treatment efficacy [29]. Specifically, inhibiting IL-6 activity and COX-2 expression may reduce cancer-triggered inflammation and enhance the body's ability to combat cancer cells. Additionally, strategies that boost anti-inflammatory cytokines such as IL-10 could restore immune balance and improve immune defense against infections. Combining these immune-modulators with conventional treatments like chemotherapy and immunotherapy could overcome resistance and lead to better patient outcomes [30]. Targeting inflammation within cervical tumors could therefore enhance the success of existing therapies and aid in the development of personalized medicine, improving prognosis and survival rates in clinical practice.

In addition to identifying prognostic markers linked to inflammation-related gene signatures, it is important to understand how these genes mediate their effects in the TME of cervical cancer. Key regulators of immune responses and TME structure, such as MMP-9, IL-6, COX-2, TNF-α, and IL-10, contribute to tumor progression and immune evasion. The matrix metalloproteinase MMP-9, a protease that degrades the extracellular matrix, facilitates tumor cell invasion and metastasis, while also creating an environment conducive to immune cell infiltration, potentially leading to ineffective immune responses [31]. IL-6, a pro-inflammatory cytokine, stimulates multiple signaling cascades, promoting tumor cell proliferation and survival by mimicking the JAK/STAT pathway. It also recruits immunosuppressive cell types, such as myeloid-derived suppressor cells and regulatory T cells, which inhibit antitumor immunity [32]. COX-2, involved in prostaglandin synthesis, plays a critical role in angiogenesis, which supports tumor growth by providing nutrients and oxygen. Furthermore, COX-2 suppresses the cell-mediated immune response, enabling tumor evasion of immune destruction [33]. TNF-α, another inflammatory cytokine, plays a dual role in tumor growth, promoting inflammation and immune cell recruitment, while in some contexts, inducing tumor necrosis and cell survival [34]. IL-10, an anti-inflammatory cytokine typically downregulated in the poor prognosis group, counterbalances excessive inflammation. Lower IL-10 levels result in an uncontrolled immune response, fostering an inflammatory environment that supports tumor survival and metastasis [35]. Collectively, these inflammatory mediators reshape the TME, facilitating tumor progression and hindering effective immune responses, making them valuable therapeutic targets for improving cervical cancer treatment strategies.

While our study supports the predictive value of these biomarkers, there are limitations to our research. A major drawback of the retrospective design is the potential for biases, such as selection bias and confounding, which may hinder the generalizability of the findings to other populations. Furthermore, the relatively small sample size limits the statistical power and robustness of the results, potentially reducing the precision of the estimated effects and increasing the risk of overfitting in the predictive model. This limitation underscores the importance of cautious interpretation and highlights the need for validation in larger, independent cohorts. Additionally, using peripheral blood mononuclear cells to study gene expression, rather than tumor tissue, may not fully capture the tumor's specific inflammatory signals. To refine and validate the model, further research utilizing biopsy samples and larger, well-powered prospective studies is essential. While the biomarkers identified in this study are significant, their clinical application requires confirmation through studies involving larger and more diverse populations to ensure scientific rigor and broader applicability.

In conclusion, this study provides a comprehensive analysis of inflammation-related gene signatures as prognostic biomarkers in cervical cancer. By integrating these biomarkers with clinical features, we developed a nomogram model that enhances risk stratification and offers a more personalized approach to treatment. The findings underscore the importance of inflammation in cervical cancer progression and suggest that targeting inflammatory pathways could serve as a novel therapeutic strategy. Despite the study's limitations, the results hold promise for improving prognosis and management of cervical cancer, particularly through the use of inflammation-targeted therapies.

Disclosure of conflict of interest

None.

Abbreviations

MMPs, Matrix Metalloproteinases; TNF-α, Tumor Necrosis Factor Alpha; IL-6, Interleukin 6; IL-1β, Interleukin 1 Beta; NF-κB, Nuclear Factor Kappa B; CXCL8, C-X-C Motif Chemokine Ligand 8; IL-10, Interleukin 10; COX-2, Cyclooxygenase 2; MMP-9, Matrix Metalloproteinase 9; TGF-β1, Transforming Growth Factor Beta 1; CRP, C-Reactive Protein; FIGO, The International Federation of Gynecology and Obstetrics; PBMCs, Peripheral Blood Mononuclear Cells; qPCR, Quantitative Polymerase Chain Reaction; CIs, Confidence Intervals; HRs, Hazard Ratios; DCA, Decision Curve Analysis; HPV, Human Papillomavirus; ROC, Receiver Operating Characteristic.

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