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Original Article

Clinical significance of MIF in predicting lymph node metastasis and immune-inflammatory characteristics in endometrial cancer: a retrospective study of 361 patients

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Abstract: Background: Macrophage migration inhibitory factor (MIF) contributes to the progression of diverse malignancies. However, its expression and relation with systemic immune-inflammatory characteristics in predicting lymph node metastasis (LNM) in endometrial cancer (EC) remains unclear. Objective: The current study evaluates the association between MIF-related inflammatory features and LNM, and establishes a preoperative model for predicting LNM in EC. Methods: The current study enrolled 361 EC patients, and their clinical characteristics, hematologic parameters, tumor biomarkers, inflammatory indices, imaging features, and preoperative pathological findings were collected. MIF expression and relevant immune-inflammatory indicators were analyzed for their correlations with LNM. Independent predictors were identified using multivariable logistic regression. Model performance was assessed using ROC curves, calibration plots, and decision curve analysis (DCA). Results: Among the 361 patients, LNM occurred in 51 patients (15.8%). Patients with LNM had significantly higher levels of CA125, HE4, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and larger tumor diameter. Myometrial invasion ≥1/2, cervical stromal involvement, and non-endometrioid histology were all significantly more frequent in the LNM group. Multivariate logistic regression identified high MIF expression, SII (per 100 units), myometrial invasion ≥50%, LVSI, and high-grade histology as independent predictors of LNM. The nomogram demonstrated excellent discriminatory performance, good calibration, and favorable clinical utility on decision curve analysis. Conclusion: Tumor MIF expression is an independent predictor of LNM in EC. Incorporation of MIF into a clinically grounded prediction model may enhance preoperative risk stratification of EC.

Keywords: Macrophage migration inhibitory factor, endometrial cancer, lymph node metastasis, immune-inflammatory indices, nomogram

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in many high-income countries, and its global incidence and mortality continue to rise in parallel with aging populations and increasing obesity rates [1]. Although most women present with apparently early-stage disease and have favorable outcomes after surgery, a substantial subset harbor occult lymph node metastasis (LNM) or exhibit aggressive biological behavior that leads to recurrence and death. Contemporary staging systems and management guidelines, including the revised International Federation of Gynecology and Obstetrics (FIGO) classification [2] and European Society of Gynaecological Oncology (ESGO), European Society for Radiotherapy and Oncology (ESTRO), and European Society of Pathology (ESP) recommendations, underscore LNM as a pivotal determinant of risk grouping, adjuvant treatment decisions, and prognosis in EC [3].

However, the optimal extent of lymph node assessment remains controversial. Systematic pelvic and para-aortic lymphadenectomy improves staging accuracy but has not consistently translated into survival benefit and is associated with lymphedema, lymphocyst formation,

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and operative morbidity [2]. Sentinel lymph node mapping reduces surgical trauma, yet it is not universally available and still carries procedure-related risks. These uncertainties have driven intense interest in preoperative tools that can stratify the risk of LNM and tailor nodal surgery. Several groups have proposed clinicopathologic or metabolic-risk-based nomograms. For example, Feng et al. integrated a metabolic syndrome score with tumor characteristics to predict LNM in EC, achieving reasonable discriminative power [4]. More recently, Lin et al. developed a metabolic-inflammatory-nutritional score (MINS) and showed that it was independently associated with both LNM and survival, supporting the concept that systemic host status and tumor spread are tightly linked [5]. Nonetheless, these models do not explicitly incorporate tumor-derived immune mediators, and their biological underpinnings remain incompletely understood.

Accumulating evidence indicates that systemic inflammation-based indices derived from peripheral blood counts, such as the systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and composite nutritional-inflammatory scores, have prognostic relevance in EC. Lei et al. first demonstrated that preoperative SII is an independent predictor of LNM in EC, suggesting that heightened systemic inflammation reflects a microenvironment permissive for nodal dissemination [6]. Matsubara et al. reported that elevated pre-treatment SII was associated with shorter progression-free and overall survival, outperforming NLR and PLR as a survival marker [7]. In the postoperative setting, Huang et al. found that high SII remained an adverse prognostic factor after curative surgery [8]. Prospective and multicenter cohorts have further shown that pre-treatment inflammatory parameters, such as C-reactive protein (CRP), monocyte-to-lymphocyte ratio, and composite scores like the hemoglobin-albumin-lymphocyte-platelet (HALP) index, are associated with survival in EC and may refine risk stratification beyond conventional clinicopathologic factors [9, 10]. Despite these advances, systemic indices are indirect readouts of the host-tumor interaction and do not specify which cytokines or signaling axes drive both immune dysregulation and metastatic spread.

Macrophage migration inhibitory factor (MIF) is a pleiotropic, evolutionarily conserved cytokine that has emerged as a central regulator of innate and adaptive immunity [11]. Unlike many other stress hormones, MIF counteracts the anti-inflammatory effects of glucocorticoids and sustains pro-inflammatory signaling through pathways such as MAPK, PI3K-AKT, and NF-κB [11]. Clinical and experimental studies across diverse diseases have shown that MIF is elevated in inflamed tissues and circulation, and that its levels correlate with disease severity. Grieb et al. systematically reviewed clinical data and proposed MIF as a promising biomarker in several inflammatory and neoplastic conditions [12]. These features position MIF as a candidate molecular bridge between systemic inflammation and local tumor biology.

In oncology, MIF is increasingly recognized as a driver of tumor initiation, progression, and immune escape. Wang et al. showed that MIF overexpression in head and neck squamous cell carcinoma promoted tumor growth and was associated with advanced stage and poor outcome, leading the authors to describe MIF as a “potential driver and biomarker” in this setting [13]. Functional studies have demonstrated that MIF protects cancer cells from immunogenic cell death and dampens anti-tumor immune responses. Balogh et al. reported that genetic or pharmacologic MIF inhibition enhanced the efficacy of immunogenic chemotherapy in a murine colon cancer model [14]. Regarding its role in the microenvironment, MIF contributes to an immunosuppressive niche by skewing tumor-associated macrophages (TAMs) toward an alternatively activated, pro-tumor phenotype [15]. Collectively, the previous studies support a model in which high MIF expression sustains chronic inflammation, drives angiogenesis and invasion, and simultaneously impairs anti-tumor immunity.

Regarding EC, data on MIF are still limited but intriguing. Xiao et al. analyzed MIF and c-erbB-2 expression in normal endometrium, hyperplasia, and EC, and found stepwise upregulation of MIF protein and mRNA from benign to malignant lesions [16]. Their study suggested that MIF overexpression is associated with EC occurrence and with certain clinicopathologic features, but the sample size was modest, and the findings regarding invasive behavior and LNM were not definitive. Other small immunohistochemical series have similarly reported higher MIF expression in EC compared with normal or hyperplastic endometrium, yet they

rarely incorporated comprehensive lymph node evaluation, systemic inflammatory indices, or detailed survival endpoints. As a result, it remains unclear whether tumor MIF expression mirrors or modulates host systemic inflammation, whether it independently predicts LNM, and how it integrates with established risk factors and blood-based inflammatory scores in EC.

Given that LNM is central to the progression of EC, there is a need to identify biologically grounded markers that complement established pathological risk factors. Although MIF has been conceived as a key tumor-derived cytokine implicated in tumor-associated inflammation, immune evasion, and metastatic progression across multiple solid malignancies, its clinical relevance in EC remains insufficiently defined. In the current retrospective study, we analyzed a cohort of 361 patients with pathologically confirmed endometrioid EC and quantified the levels of MIF by immunohistochemistry (IHC). The associations of MIF with LNM, systemic immune-inflammatory indices (SII), and clinicopathological features were evaluated. In addition, rather than proposing an alternative to established predictors such as lymphovascular space invasion and tumor grade, the current study aims to determine whether MIF provides independent and complementary predictive value when integrated with systemic inflammatory burden and conventional pathological factors. By incorporating a tumor-derived inflammatory mediator into a clinically grounded prediction framework, the present work seeks to refine preoperative risk stratification for LNM and to clarify the clinical significance of MIF in the diagnosis and prognosis of EC.

Methods

Study design and population

The current analysis firstly enrolled 412 patients who were retrospectively reviewed and underwent primary surgical treatment for histologically confirmed endometrial cancer at the Affiliated Hospital of Hebei University of Engineering between January 2023 and December 2024. Afterwards, patients were excluded if they met any of the following criteria: 1) Receipt of any neoadjuvant chemotherapy, radiotherapy, or hormonal therapy prior to surgery. 2) History of other synchronous or previous malignancies. 3) Presence of acute or chronic infectious, autoimmune, or hematologic diseases that could influence systemic inflammatory indices. 4) Use of systemic immunosuppressive agents or glucocorticoids within one month before surgery. 5) Incomplete clinicopathological, laboratory, or lymph node assessment data. 6) Unavailability of adequate formalin-fixed, paraffin-embedded tumor tissue for immunohistochemical evaluation of MIF. After applying these criteria, 361 patients with complete clinical, pathological, laboratory, and immunohistochemical data were finally included in the analysis cohort. Based on postoperative pathological examination of resected lymph nodes, patients were classified as lymph node metastasis-positive (LNM+), and lymph node metastasis-negative (LNM-). No strict sample size was set up in the analysis due to its retrospective design, and the study collected as much patient information as possible.

Clinical, laboratory, and imaging data collection

Preoperative serum tumor markers and hematological parameters were collected within seven days before surgery. Fasting venous blood (3-5 mL) was drawn into serum separation tubes, centrifuged at 3,000×g for 10 minutes at 4°C, and analyzed immediately for CA125, HE4, CA19-9, CA15-3, and carcinoembryonic antigen (CEA) using an automated chemiluminescent immunoassay analyzer (Roche Cobas e601 or equivalent), with daily calibration and dual-level internal quality control. A second blood sample (2 mL) was collected in EDTA-K2 anticoagulant tubes for complete blood count, including white blood cells, neutrophils, lymphocytes, monocytes, hemoglobin, and platelets, using a Sysmex XN-9000 analyzer; all analyses were performed within two hours of collection. Inflammatory indices were calculated using the following formulas: NLR = neutrophils/lymphocytes, PLR = platelets/lymphocytes, SII = platelets × neutrophils/lymphocytes, and systemic Inflammation Response Index (SIRI) = neutrophils × monocytes/lymphocytes. Preoperative transvaginal ultrasonography was performed using a 5-9 MHz probe with patients in lithotomy position after bladder emptying; endometrial thickness and maximal tumor diameter were measured in sagittal and transverse planes, recorded three

times, and averaged. Pelvic MRI was conducted using a 1.5 T or 3.0 T scanner with T1-, T2-weighted, and diffusion-weighted sequences, and contrast enhancement when indicated; radiologists evaluated the depth of myometrial invasion, cervical stromal involvement, and suspicious lymphadenopathy independently and were blinded to pathological results.

Pathological examination

Surgical specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for routine assessment. Two senior pathologists independently evaluated histologic type (endometrioid or non-endometrioid), histologic grade (G1-G3), depth of myometrial invasion, cervical stromal involvement, lymphvascular space invasion (LVSI), and the number of retrieved and metastatic lymph nodes. Discrepancies were resolved by joint review to ensure diagnostic consistency.

Immunohistochemistry (IHC) detections

The expression level of tumor MIF protein expression was evaluated using immunohistochemistry (IHC) detection: briefly, tumor samples were subjected to formalin-fixation and paraffin-embedded, and then 4-μm tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval using citrate buffer). Endogenous peroxidase activity was blocked, followed by incubation with a primary anti-MIF antibody at 4°C overnight. The expression of MIF was semi-quantitatively assessed using the H-score method, detailed as follows, by two senior pathologists blinded to the study design: H-score = Σ (percentage of positively stained tumor cells × staining intensity score), and staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong), yielding a total score ranging from 0 to 300. For analytical purposes, patients were dichotomized into high-MIF and low-MIF expression groups with the median H-score as the cutoff value.

Tumor immune microenvironment markers, including CD68 (pan-macrophages), CD163 (M2 macrophages), CD8 (cytotoxic T lymphocytes), and myeloperoxidase (MPO; neutrophils), were also detected using IHC as described. All slides were independently evaluated by two experienced pathologists who were blinded to clinical data, MIF expression status, and lymph node metastasis, and discrepancies were resolved by consensus review. The levels of immune microenvironment markers were analyzed as exploratory variables to characterize the immunological context associated with different levels of tumor MIF expression, and thus not included as candidate predictors for the multivariable clinical prediction model.

Outcome measures

The primary outcome was the presence of lymph node metastasis determined by postoperative pathology. Secondary outcomes included tumor MIF protein expression and its associations with immune microenvironment characteristics, systemic immune-inflammatory indices, tumor markers, and clinicopathological features.

Statistical analysis

All statistical analyses were conducted using SPSS 26.0 and R 4.3.2. Continuous variables were tested for normality using the Shapiro-Wilk test and expressed as mean ± standard deviation or median (interquartile range) according to data distribution. Differences between-groups were analyzed using the independent-samples t-test or Mann-Whitney U test. Categorical variables were analyzed using the χ^2 test or Fisher's exact test. Spearman correlation was performed to assess relationships between variables. Continuous variables, including systemic inflammatory indices such as SII, NLR, PLR, LMR, and PNI, were analyzed primarily as continuous predictors. For regression interpretability, SII was scaled by 100 units and entered into the logistic regression model as SII (per 100-unit increase). Logistic regression analysis was conducted to identify independent predictors of LNM, and variables showing $P<0.10$ in univariate analysis were included in multivariate models. A nomogram was constructed then based on the regression. Internal validation of the prediction model was performed using bootstrap resampling with 1,000 iterations to evaluate model stability and reduce optimism bias. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC). Calibration was evaluated by comparing predicted and observed probabilities using bootstrap-corrected calibration curves. Clinical util-

Table 1. Baseline clinicopathological characteristics and immune-inflammatory markers in LNM- vs. LNM+ groups

Variable	Total (n = 361)	LNM- (n = 304)	LNM+ (n = 57)	Test statistic	*P* value
Age, years (mean ± SD)	57.1 ± 9.4	56.8 ± 9.2	58.9 ± 10.1	t = -1.47	0.142
BMI, kg/m^2	26.1 ± 3.8	26.0 ± 3.7	26.7 ± 4.1	t = -1.24	0.215
Menopause (%)	228 (63.2%)	187 (61.5%)	41 (71.9%)	χ^2 = 2.16	0.142
High-grade (G3) (%)	98 (27.1%)	66 (21.7%)	32 (56.1%)	χ^2 = 29.94	<0.001
Non-endometrioid histology (%)	29 (8.0%)	15 (4.9%)	14 (24.6%)	χ^2 = 23.61	<0.001
Tumor size (cm, median IQR)	2.5 (1.8-3.5)	2.3 (1.7-3.1)	3.6 (2.5-4.8)	Z = -5.86	<0.001
Myometrial invasion ≥50% (%)	118 (32.7%)	80 (26.3%)	38 (66.7%)	χ^2 = 34.61	<0.001
Cervical stromal invasion (%)	45 (12.5%)	26 (8.6%)	19 (33.3%)	χ^2 = 28.04	<0.001
LVSI (%)	85 (23.5%)	46 (15.1%)	39 (68.4%)	χ^2 = 78.12	<0.001
CA125 (U/mL, median IQR)	23.8 (14.9-40.7)	21.5 (14.2-34.5)	55.6 (29.8-92.4)	Z = -6.72	<0.001
SII	612 (410-890)	550 (380-790)	1020 (780-1460)	Z = -7.01	<0.001
NLR	2.48 (1.78-3.35)	2.32 (1.70-3.10)	3.76 (2.88-5.12)	Z = -6.84	<0.001
PLR	149 (102-208)	142 (98-196)	226 (168-310)	Z = -6.59	<0.001
LMR	2.91 (2.20-3.75)	3.04 (2.38-3.89)	1.88 (1.40-2.55)	Z = -6.77	<0.001
PNI	50.8 ± 5.2	51.4 ± 5.1	47.6 ± 4.8	t = 5.32	<0.001

BMI, body mass index; LVSI, lymph-vascular space invasion; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocytes-to-monocytes ratio; PNI, prognostic nutritional index. Continuous variables are presented as mean ± standard deviation when normally distributed and as median (interquartile range) when non-normally distributed. Between-group comparisons were performed using the independent-samples t test or Mann-Whitney U test, as appropriate.

ity was assessed using decision curve analysis (DCA) by quantifying the net benefit across a range of threshold probabilities. External validation was not performed in this study because an independent cohort with comparable clinicopathological and immunohistochemical data was not available. To increase the robustness of the predictive model, a sensitivity analysis using propensity score matching (PSM) strategy was performed, which could minimize the potential impact of baseline imbalance between the LNM+ and LNM- groups. Propensity scores were estimated using a logistic regression model including age, body mass index, histological type, tumor grade, tumor size, depth of myometrial invasion, lymph-vascular space invasion (LVSI), and CA125 level. Patients were matched using a 1:1 nearest-neighbor matching algorithm without replacement, with a caliper width of 0.2 of the standard deviation of the logit of the propensity score. Covariate balance before and after matching was evaluated using standardized mean differences (SMDs), with values <0.1 indicating adequate balance. Logistic regression analysis and model performance evaluation were repeated in the matched cohort as a sensitivity analysis. Statistical significance was defined as P<0.05.

Results

Baseline characteristics of the study population

A total of 361 patients with endometrial cancer were included, of whom 57 (15.8%) had lymph node metastasis (LNM+). Baseline characteristics are summarized in **Table 1**: compared with LNM- patients, those with LNM+ exhibited significantly more aggressive pathological features, including a higher proportion of high-grade tumors (56.1% vs. 21.7%, P<0.001), non-endometrioid histology (24.6% vs. 4.9%, P<0.001), larger tumor size, deeper myometrial invasion (66.7% vs. 26.3%, P<0.001), cervical stromal invasion (33.3% vs. 8.6%, P<0.001), and LVSI (68.4% vs. 15.1%, P<0.001). Serum CA125 levels were also significantly higher in the LNM+ group (P<0.001).

Systemic inflammatory markers differed markedly between groups (**Table 1**). Patients with LNM+ had substantially elevated SII, NLR, and PLR, as well as lower lymphocyte-to-monocyte ratio (LMR) and prognostic nutritional index (PNI), indicating a heightened systemic inflammatory status associated with nodal spread (all P<0.001).

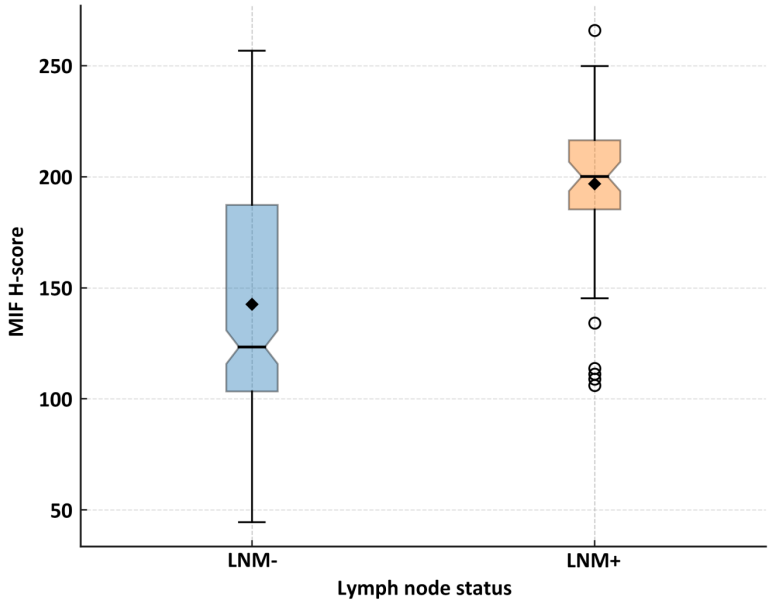

Figure 1. MIF expression according to lymph node status (361 patients, comprising 57 LNM+ patients and 304 LNM- patients). Median MIF expression was significantly higher in LNM+ tumors, demonstrating a strong association between MIF overexpression and metastatic spread. The black diamond represents the mean value, and the horizontal line within each box indicates the median. Continuous variables were compared using the Mann-Whitney U test. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate.

MIF expression and its association with lymph node metastasis

As shown in **Figure 1**, MIF H-scores were significantly higher in tumors from LNM+ patients compared with LNM- patients (median ≈ 200 vs. 120, P<0.001). High-MIF expression was also strongly associated with multiple aggressive pathological characteristics, including high-grade histology (41.1% vs. 13.3%), deeper myometrial invasion (42.8% vs. 22.7%), and LVSI (34.4% vs. 12.7%), all P<0.001 (**Table 2**). Notably, the rate of lymph node metastasis in patients with high MIF expression (25.6%) was more than four times that of patients with low MIF (6.1%), underscoring the strong association between MIF overexpression and metastatic risk.

Tumor immune microenvironment differences between low- and high-MIF groups

Significant immune microenvironment differences were observed between MIF groups (**Figure 2**; **Table 2**). High-MIF tumors exhibited increased infiltration of CD68+ macrophages, CD163+ M2 macrophages, and MPO+ neutrophils, indicating a pro-inflammatory and pro-tumorigenic milieu. Conversely, CD8+ T-cell density was significantly reduced in high-MIF tumors compared with low-MIF tumors (median ≈ 38 vs. 68 cells/HPF, P< 0.001). These findings support the role of MIF in shaping an immunosuppressive tumor microenvironment conducive to invasion and metastasis.

Correlations between MIF, immune cell infiltration, and systemic inflammation

Correlation analysis (**Table 3**) showed strong positive associations between MIF and CD68+ macrophages (ρ = 0.57), CD163+ M2 macrophages (ρ = 0.63), SII (ρ = 0.52), and NLR (ρ = 0.46), while MIF was negatively correlated with CD8+ T-cell infiltration (ρ = -0.49), indicating a relationship between increased MIF expression and diminished anti-tumor immunity. Moreover, MIF also positively correlated with LVSI (ρ = 0.45) and deeper myometrial invasion (ρ = 0.40), which was consistent with its association with invasive tumor behavior (**Figure 3**). The data highlighted the integrated relationship between MIF, the immune microenvironment, inflammation, and tumor aggressiveness.

Independent predictors of lymph node metastasis

Based on the univariate logistic regression, high MIF expression, elevated SII, high-grade histology, deep myometrial invasion, LVSI, and tumor size were significantly associated with LNM. In multivariate analysis (**Table 4**), five independent predictors remained, including high MIF expression (OR = 3.48, 95% CI 1.68-7.24; P = 0.001), SII (OR per 100 units = 1.14; 95% CI 1.05-1.26; P = 0.004), myometrial invasion ≥50% (OR = 3.01, 95% CI 1.42-6.25; P = 0.004), LVSI (OR = 6.78, 95% CI 3.24-14.20; P<0.001), and high-grade histology (OR = 2.11,

Table 2. MIF expression and immune microenvironment markers (Low vs. High MIF)

Variable	Low MIF (n = 181)	High MIF (n = 180)	Test statistic	*P* value
CD68+ macrophages (cells/HPF)	42 (31-56)	78 (61-102)	Z = -10.21	<0.001
CD163+ M2 macrophages (cells/HPF)	21 (16-29)	59 (44-78)	Z = -11.03	<0.001
CD8+ T cells (cells/HPF)	68 (52-92)	38 (26-55)	Z = -9.64	<0.001
MPO+ neutrophils (cells/HPF)	12 (7-19)	31 (22-46)	Z = -10.88	<0.001
High tumor grade (G3) (%)	24 (13.3%)	74 (41.1%)	χ^2 = 36.92	<0.001
Myometrial invasion ≥50% (%)	41 (22.7%)	77 (42.8%)	χ^2 = 16.98	<0.001
LVSI (%)	23 (12.7%)	62 (34.4%)	χ^2 = 24.71	<0.001
LNM (%)	11 (6.1%)	46 (25.6%)	χ^2 = 26.38	<0.001

LVSI, lymph-vascular space invasion; LNM, lymph node metastasis. Continuous variables are presented as mean ± standard deviation when normally distributed and as median (interquartile range) when non-normally distributed. Between-group comparisons were performed using the independent-samples t test or Mann-Whitney U test, as appropriate.

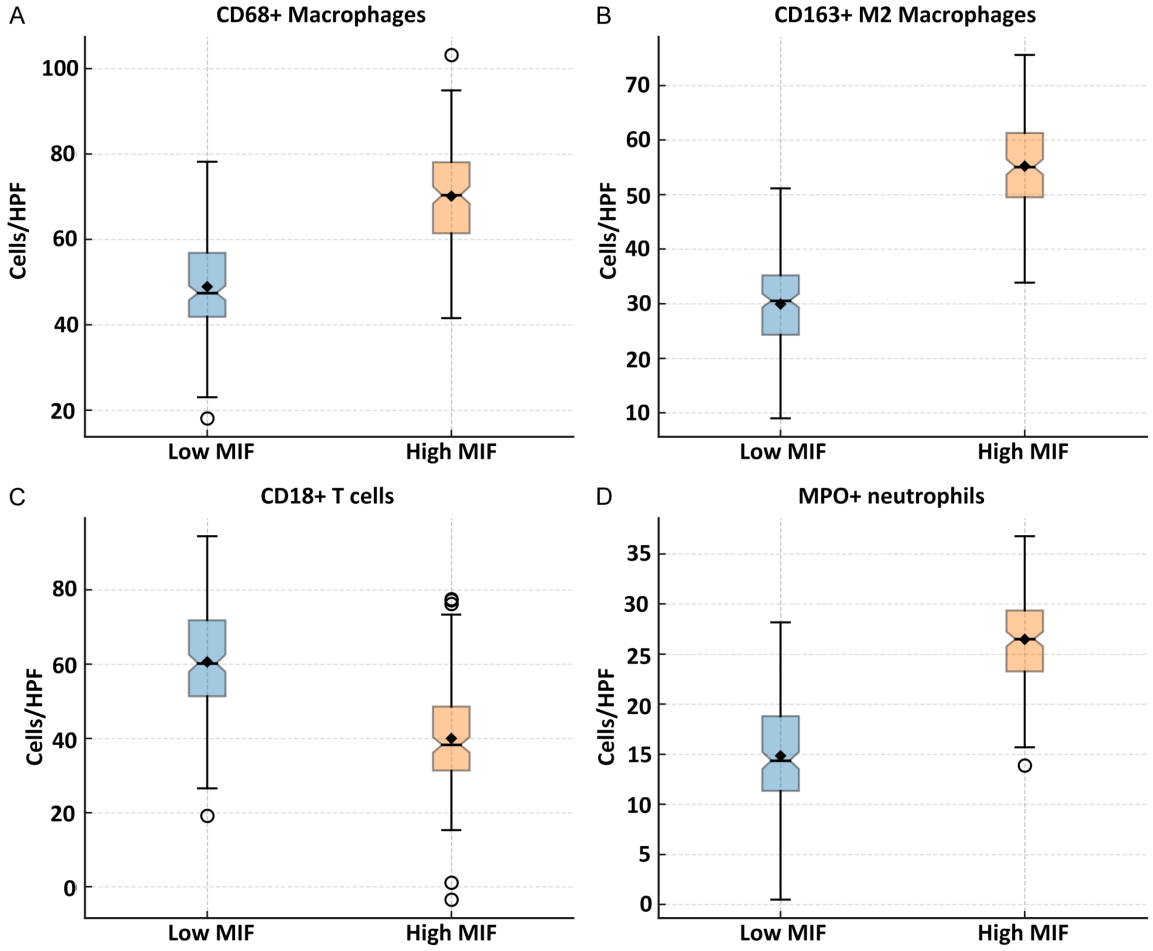

Figure 2. Tumor immune microenvironment according to MIF expression (361 patients, comprising 180 low MIF patients and 181 high MIF patients). High-MIF tumors exhibited significantly increased densities of CD68+ macrophages (A), CD163+ M2 macrophages (B), and MPO+ neutrophils (D), along with reduced CD8+ T-cell infiltration (C). Together, these shifts reflect an immunosuppressive and inflammation-driven tumor microenvironment in high-MIF disease. Means are indicated by black diamonds. Continuous variables were compared using the Mann-Whitney U test. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate.

95% CI 1.02-4.36; P = 0.043). The analysis results indicated that both MIF expression and systemic inflammation are independent contributors to nodal metastasis in EC.

Table 3. Spearman correlations between MIF levels and immune/inflammatory parameters

Variable	Spearman rho (ρ)	*P* value
MIF vs. SII	0.52	<0.001
MIF vs. NLR	0.46	<0.001
MIF vs. PLR	0.41	<0.001
MIF vs. LMR	-0.38	<0.001
MIF vs. CD68	0.57	<0.001
MIF vs. CD163 (M2)	0.63	<0.001
MIF vs. CD8+ T cells	-0.49	<0.001
MIF vs. LVSI	0.45	<0.001
MIF vs. depth of invasion	0.4	<0.001

LVSI, lymph-vascular space invasion; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocytes-to-monocytes ratio.

Nomogram development and model performance

A nomogram was constructed based on the five independent predictors (**Figure 4A**) and it demonstrated discriminatory performance with an AUC of 0.87 (95% CI, 0.82-0.92) (**Figure 4B**). The nomogram achieved an overall accuracy of 0.80, with balanced sensitivity (0.81) and specificity (0.79), reflecting a stable classification profile across risk groups. A Hosmer-Lemeshow goodness-of-fit *P* value of 0.47 indicated a moderate calibration without significant deviation between predicted and actual LNM rates. In addition to the Hosmer-Lemeshow test, quantitative calibration metrics were further assessed. The calibration-in-the-large value was close to zero, indicating minimal systematic over- or under-estimation of lymph node metastasis risk, while the calibration slope was close to 1, suggesting excellent agreement between predicted and observed probabilities across the full risk spectrum. Internal validation with 1,000 bootstrap resamples yielded a bootstrap-corrected AUC of 0.85, demonstrating minimal optimism and supporting the robustness and generalizability of the predictive model. Furthermore, calibration analysis confirmed strong agreement between predicted and observed probabilities of LNM (**Figure 5A**) and DCA (**Figure 5B**) indicating that the model provided superior net clinical benefit over "treat-all" and "treat-none" strategies across a wide range of threshold probabilities (0.10-0.70), demonstrating its clinical utility for individualized surgical decision-making.

Sensitivity analysis after PSM

The robustness of the predictive model was further assessed using PSM strategy to minimize the potential bias introduced by the imbalance in sample size. The analysis was performed using a multivariable logistic regression model incorporating age, body mass index, histological type, tumor grade, tumor size, depth of myometrial invasion, LVSI, and CA125 level. Patients were matched at a 1:1 ratio using nearest-neighbor matching without replacement, with a caliper width of 0.2 of the standard deviation of the logit of the propensity score.

After matching, 57 patients with lymph node metastasis (LNM+) were successfully matched with 57 patients without lymph node metastasis (LNM-). Baseline clinicopathological characteristics were well balanced between the two groups, with all standardized mean differences (SMDs) below 0.1, indicating adequate covariate balance (**Table 5**). In the matched cohort, logistic regression analysis demonstrated that high MIF expression remained significantly associated with lymph node metastasis. In addition, elevated SII, deep myometrial invasion (≥50%), and LVSI were also identified as independent predictors of LNM, whereas tumor grade did not retain statistical significance after adjustment (**Table 6**). These findings were consistent with those observed in the original cohort, supporting the stability and robustness of the proposed predictive model.

Discussion

The current study retrospectively analyzed the data from 361 EC patients, and found that high MIF expression, SII, myometrial invasion ≥50%, LVSI, and high-grade histology were independently associated with LNM. Importantly, these findings indicate that MIF and host SII contribute additional, independent information beyond established pathological risk factors, rather than replacing them. Together, these results underscore the intertwined roles of inflammatory activation, immune dysregulation, and aggressive local tumor biology in the metastatic progression of EC.

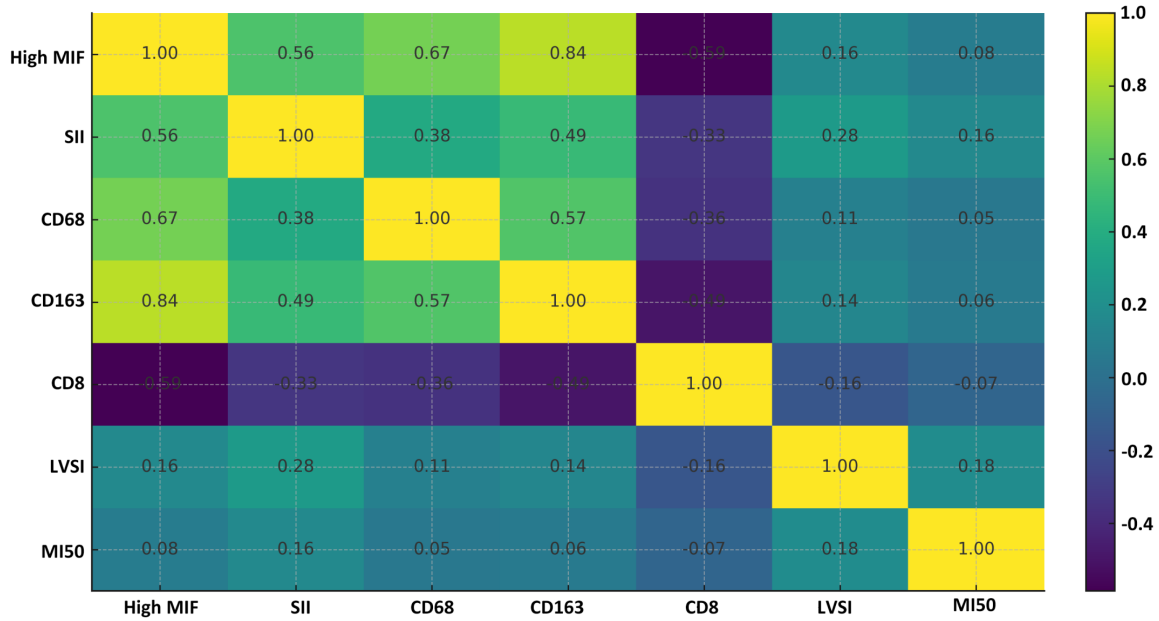

Figure 3. Correlation heatmap of MIF, systemic inflammation, immune infiltration, and pathological invasiveness. Correlation matrix displaying Spearman correlation coefficients among MIF expression, systemic inflammatory markers (SII), immune-cell infiltration markers (CD68, CD163, CD8), and pathological features (LVSI, myometrial invasion). Positive correlations are shown in warmer colors, and negative correlations in cooler colors. Values within each cell represent the correlation coefficient.

Table 4. Logistic regression for predictors of lymph node metastasis

Variable	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
High MIF expression	5.32 (2.77-10.21)	<0.001	3.48 (1.68-7.24)	0.001
SII (per 100 increase)	1.22 (1.14-1.32)	<0.001	1.14 (1.05-1.26)	0.004
High-grade (G3)	4.62 (2.57-8.31)	<0.001	2.11 (1.02-4.36)	0.043
Myometrial invasion ≥50%	5.89 (3.28-10.57)	<0.001	3.01 (1.42-6.25)	0.004
LVSI	12.79 (6.61-24.75)	<0.001	6.78 (3.24-14.20)	<0.001
Tumor size (per 1 cm)	1.61 (1.30-2.00)	<0.001	1.19 (0.94-1.51)	0.148
Non-endometrioid histology	6.10 (2.79-13.35)	<0.001	2.40 (0.95-6.01)	0.063

SII, systemic immune-inflammation index; LVSI, lymph-vascular space invasion. SII was entered as a continuous variable scaled per 100-unit increase. Inflammatory indices sharing overlapping components were evaluated individually in univariate analysis, and only SII was retained for multivariable modeling. Variables with P>0.05 in multivariable analysis were not considered independent predictors.

Our findings are consistent with previous studies demonstrating a stepwise increase in MIF expression from normal endometrium to atypical hyperplasia and EC, indicating that MIF participates in disease progression from early tumorigenesis to advanced stages [16]. Xiao et al. reported that MIF positivity increased from 20% in normal tissue to 70% in EC and was significantly associated with LNM, supporting our finding that higher circulating inflammatory load correlates with metastatic potential [16]. Additionally, our study extended these observations by demonstrating that tumor MIF expression remains independently associated with LNM, even after adjustment for established pathological risk factors and systemic inflammatory burden. Mechanistic studies in other solid tumors reinforce this relationship: MIF stimulates ERK/MAPK and PI3K/Akt activity, enhances angiogenesis, stabilizes HIF-1α under hypoxia, suppresses p53-mediated apoptosis, and promotes EMT; all of which are known to facilitate tumor invasion and nodal spread [13, 17-20]. In the context of our cohort, these mechanisms offer a coherent explanation for the observed links between high MIF expres-

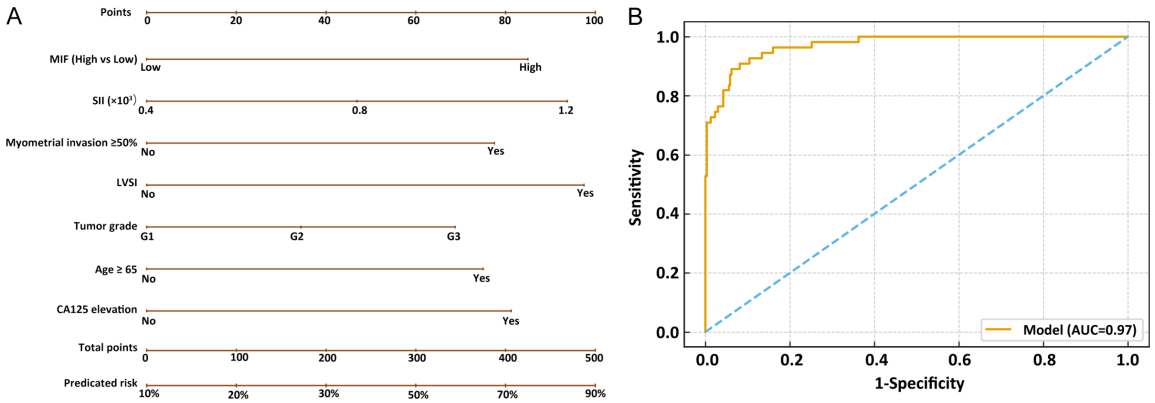

Figure 4. Nomogram for predicting lymph node metastasis and model discrimination. Schematic representation of the nomogram incorporating five independent predictors-high MIF expression, SII (per 100 units), myometrial invasion ≥50%, LVSI, and high-grade histology as independent predictors of LNM. Higher total points correspond to increased predicted probability of LNM. Each predictor corresponds to a specific score range displayed on the "Points" axis at the top of the nomogram. The total score is calculated by summing the individual scores for all variables and is shown on the "Total Points" axis (range: 0-500). The total score is then projected downward to estimate the predicted probability of lymph node metastasis, which ranges from approximately 10% to 90% on the "Predicted Risk" axis (A). Receiver operating characteristic (ROC) curve of the prediction model demonstrating excellent discrimination (AUC = 0.87) (B).

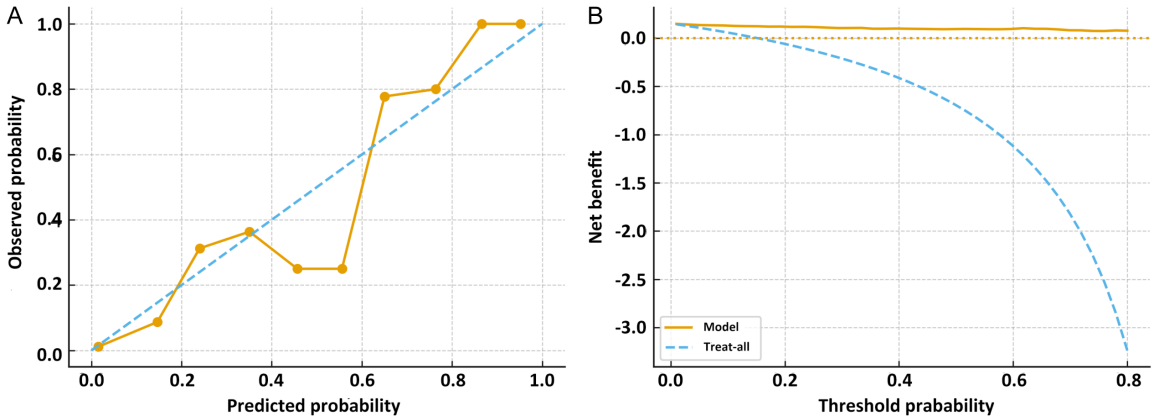

Figure 5. Internal validation of the prediction model using calibration and decision curve analyses of the predictive model. Calibration plot showing close agreement between predicted and observed lymph node metastasis probabilities, indicating good model calibration (A). Decision curve analysis demonstrating that the nomogram provides greater net clinical benefit than "treat-all" or "treat-none" strategies across a wide threshold probability range (0.10-0.70) (B).

sion, an immunosuppressive and inflammation-dominant tumor microenvironment, elevated systemic inflammatory indices, and increased risk of nodal spread.

Emerging evidence highlights the central role of MIF in remodeling the tumor immune microenvironment (TME) as a pleiotropic cytokine that drives chronic inflammation, recruits immunesuppressive myeloid populations, and orchestrates immune evasion [11, 21, 22]. Our findings provide cohort-specific support for this paradigm in EC, enabling broad modulation of myeloid and lymphoid cell function, altering dendritic-cell maturation, inhibiting cytotoxic T-cell responses, and fostering an immunosuppressive milieu [11, 21, 22]. Consistent with these mechanisms, we observed that patients with LNM exhibited elevated neutrophil- and monocyte-driven inflammatory indices together with relative lymphocyte depletion, reflecting a state of heightened systemic inflammation

Table 5. Baseline clinicopathological characteristics before and after PSM analysis

Variable	Before PSM LNM- (n = 304)	Before PSM LNM+ (n = 57)	After PSM LNM- (n = 57)	After PSM LNM+ (n = 57)	SMD (After)
Age, years (mean ± SD)	56.8 ± 9.2	58.9 ± 10.1	58.3 ± 9.6	58.9 ± 10.1	0.06
BMI, kg/m^2 (mean ± SD)	26.0 ± 3.7	26.7 ± 4.1	26.4 ± 3.9	26.7 ± 4.1	0.07
High-grade (G3) (%)	21.7%	56.1%	52.6%	56.1%	0.08
Tumor size, cm (median IQR)	2.3 (1.7-3.1)	3.6 (2.5-4.8)	3.4 (2.4-4.6)	3.6 (2.5-4.8)	0.05
Myometrial invasion ≥50% (%)	26.3%	66.7%	63.2%	66.7%	0.06
LVSI (%)	15.1%	68.4%	64.9%	68.4%	0.07
CA125 (U/mL, median IQR)	21.5 (14.2-34.5)	55.6 (29.8-92.4)	51.8 (28.9-89.6)	55.6 (29.8-92.4)	0.04

BMI, body mass index; LVSI, lymph-vascular space invasion; SMD, standardized mean difference.

Table 6. Logistic regression analysis for predictors of lymph node metastasis in the PSM cohort

Variable	Odds ratio (95% CI)	*P* value
High MIF expression	3.21 (1.42-7.05)	0.005
SII (per 100 increase)	1.16 (1.03-1.30)	0.013
Myometrial invasion ≥50%	2.84 (1.21-6.68)	0.017
LVSI	5.92 (2.41-14.56)	<0.001
Tumor grade (G3)	1.76 (0.82-3.81)	0.146

LVSI, lymph-vascular space invasion.

accompanied by compromised adaptive immunity. These systemic immune-inflammatory patterns parallel the local immune alterations associated with high tumor MIF expression, supporting a biologically coherent link between MIF-driven immune remodeling and lymphatic dissemination in endometrial cancer. Evidence from other gynecologic cancers corroborates these findings. In cervical squamous cell carcinoma, MIF promotes inflammasome activation, macrophage recruitment, and M2 polarization, thereby supporting tumor progression and metastasis [20]. Similar tumor-promoting roles have been demonstrated in gastric cancer [23]. Together with our cohort data, this cross-tumor evidence supports the concept that MIF-mediated inflammatory and immune remodeling represents a conserved pathway contributing to nodal dissemination.

Within the context of EC, our findings also parallel existing predictive-modeling studies. Both Chinese preoperative prediction models included in our dataset identified CA125, tumor diameter, deep myometrial invasion, cervical stromal invasion, and histologic type as independent predictors of LNM [24, 25]. Systemic inflammatory indices have also been shown to predict aggressive pathology and LNM in EC [8]. Based on these previous findings, the present study demonstrates that tumor MIF expression provides independent and complementary predictive value when evaluated alongside SII and established pathological risk factors. Rather than replacing conventional predictors, integrating a tumor-derived inflammatory mediator with host inflammatory status and pathological features offers a more biologically grounded framework for preoperative risk stratification of lymph node metastasis.

Clinically, tumor MIF expression and MIF-associated SII may serve as complementary biomarkers to refine preoperative risk assessment for lymph node metastasis, especially in early-stage EC where decisions about lymphadenectomy remain controversial. Traditional imaging modalities provide inconsistent sensitivity for LNM, and biomarkers such as CA125 lack specificity. Integrating immune-inflammatory markers within a clinically grounded framework may therefore improve risk stratification without additional invasive procedures. Second, the identification of high MIF expression in conjunction with elevated systemic inflammatory burden may help delineate patients who warrant more comprehensive nodal evaluation or closer postoperative surveillance, rather than directly dictating treatment escalation. Third, although therapeutic implications remain exploratory, the observed associations between MIF, immune suppression, and inflammatory activation support further investigation of MIF-related signaling pathways as potential therapeutic targets. Preclinical studies suggest that inhibition of MIF signaling can reduce tumor proliferation, angiogenesis, and

macrophage-mediated immune suppression while enhancing apoptotic sensitivity [23, 26]. In addition to discrimination performance, good model calibration is of critical clinical importance. Accurate agreement between predicted and observed probabilities ensures reliable absolute risk estimation at the individual patient level, which is essential for preoperative decision-making in endometrial cancer. A well-calibrated model allows clinicians to more confidently stratify patients according to lymph node metastasis risk, thereby informing individualized surgical planning, such as the extent of lymphadenectomy, as well as postoperative surveillance strategies. Beyond discrimination and calibration performance, the robustness of the model was further evaluated through sensitivity analysis with PSM strategy, and the results showed that the persistence of MIF as an independent predictor of LNM and the stable model performance in the matched cohort support the robustness of our findings and suggest that the observed associations are not driven by sample size disparity. However, the clinical relevance of such strategies in EC requires validation in prospective and mechanistic studies.

Despite its strengths, this study has limitations. First, this was a single-center retrospective study, and external validation in independent cohorts was not performed, which limits the immediate generalizability of the prediction model. Moreover, potential selection bias inherent to the single-center retrospective design exists, which may limit the generalizability of our findings. Multicenter prospective studies with standardized pathological and immunohistochemical assessment are warranted to externally validate and refine the model before routine clinical application. Inflammatory markers may be influenced by chronic inflammatory or metabolic conditions that were not fully captured. Molecular classification was unavailable for all cases, limiting integration of genomic and immunologic risk factors. Second, it is noted that although MIF expression was assessed using standardized IHC procedures, semi-quantitative scoring such as H-index inevitably involves a degree of subjectivity, and inter-observer variability cannot be fully excluded. Third, molecular classification of EC was lacking due to data availability, and thus future studies integrating molecular features with immune and inflammatory markers are needed. Finally, survival outcomes were not analyzed in this study, and future work with longer follow-up and sufficient event numbers is needed to evaluate the prognostic impact of MIF and the prediction model on recurrence and overall survival. Furthermore, although our findings are biologically plausible and supported by mechanistic literature, functional studies were not conducted within this cohort. Future work should integrate transcriptomic, proteomic, and spatial-immune analyses to determine how MIF-related pathways interact with EC molecular subtypes and the immune landscape. Prospective validation of the prediction model and translational studies evaluating MIF inhibition in EC models are warranted.

In conclusion, this study demonstrates that MIF is not merely a broadly expressed inflammatory cytokine but an independent and complementary predictor of LNM in EC when integrated with systemic inflammatory burden and conventional pathological risk factors. Integrating MIF-related inflammatory markers with traditional clinical and pathological predictors may enhance the identification of high-risk patients and support more individualized treatment strategies. Given the expanding understanding of MIF's role in tumor immunity, therapeutic targeting of this pathway may ultimately contribute to improved management of aggressive EC.

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Disclosure of conflict of interest

None.

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