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Original Article

Correlation of immune-inflammatory and tumor markers with lymph node metastasis in gastric cancer

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Abstract: Objective: To evaluate the combined predictive value of immune-inflammatory and tumor markers for lymph node metastasis (LNM) in gastric cancer (GC) patients. Methods: We conducted a retrospective study of 207 GC patients who underwent radical gastrectomy. Based on postoperative histology, patients were categorized into LNM and non-LNM groups. Preoperative serologic levels of markers including carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA199), carbohydrate antigen 72-4 (CA724), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Lymphocyte-to-Monocyte Ratio (LMR), Interleukin-6 (IL-6), and C-Reactive Protein (CRP) were collected. A nomogram prediction model was developed using multivariate logistic regression. Internal validation was performed using Bootstrap resampling, and external validation was conducted on an independent cohort of 97 patients. Results: LNM was present in 55 (26.6%) patients in the training cohort. Multivariate analysis identified preoperative levels of CEA (odds ratio [OR]=1.52, P<0.001), CA724 (OR=1.24, P<0.001), NLR (OR=2.86, P<0.001), and IL-6 (OR=1.97, P<0.001) as independent risk factors for LNM. The nomogram model incorporating these four factors demonstrated excellent discrimination, with an area under the curve (AUC) of 0.93. The model significantly outperformed conventional clinicopathologic indicators (P<0.001). Good calibration and clinical utility were confirmed by calibration curves and decision curve analysis, respectively. The model maintained strong predictive performance in both internal (AUC=0.92) and external (AUC=0.91) validation cohorts. Conclusion: The combination of CEA, CA724, NLR, and IL-6 serves as an effective preoperative predictor of LNM in GC. The nomogram model based on these markers provides a reliable, non-invasive tool for individualized risk assessment and treatment planning.

Keywords: Gastric cancer, immune inflammation markers, tumour markers, lymph node metastasis

Introduction

Gastric cancer (GC) is a malignant tumor of the digestive tract with a high incidence and mortality rate worldwide [1]. According to global cancer statistics, there were more than 1.23 million new cases of GC worldwide in 2021, resulting in more than 950,000 deaths [2]. Among these, new cases of GC and GC-related deaths in China accounted for more than 40% of the global GC burden [3]. Although radical surgery combined with adjuvant chemotherapy significantly prolongs patient survival, postoperative lymph node metastasis (LNM) remains a key factor contributing to treatment failure and poor prognosis [4, 5]. Studies show that the incidence of LNM after surgery in patients with early-stage GC is around 10% to 20%, but in patients with mid- to late-stage GC, it may exceed 40% or even worse [6-8]. Therefore, exploring reliable predictive indicators to identify high-risk populations for LNM at an early stage and optimizing individual treatment strategies has become an important research direction for improving the prognosis of GC.

Although traditional pathologic staging systems provide a basic framework for assessing LNM, their reliance on postoperative results limits their predictive ability [9]. In recent years, with the deepening understanding of the tumour microenvironment, the roles of immune cell infiltration, inflammatory responses, and tumour-associated factors in tumour progression have gradually been revealed [10, 11]. The occurrence and progression of GC are the result of the dynamic interaction between tumor cells and the host immune system as well as the

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inflammatory network [12]. Immune escape, chronic inflammation, and the secretion of protumour factors can collectively create a microenvironment conducive to tumour metastasis, which has been confirmed in many studies to be closely related to the prognosis of GC [13]. GC cells can evade immune surveillance through various mechanisms, and the host's immune status may affect the invasiveness and metastatic potential of tumours [14]. In addition, systemic inflammatory responses not only contribute to tumour progression, but may also promote lymphatic metastasis by promoting angiogenesis and matrix remodeling [15]. On the other hand, some tumour-related markers can directly regulate the migration and invasion capabilities of tumour cells, thereby affecting the occurrence of LNM [16]. Therefore, the results of these serological tests can provide potential biological evidence for the prediction of LNM.

Although studies have explored the relationship between single immune, inflammatory, or tumour factors and GC prognosis, there has been a lack of systematic evaluation of the combined predictive value of multiple factors. In addition, the correlation between these markers and the clinical characteristics of GC patients (such as age, gender, tumour location, and differentiation degree) has not been fully elucidated. The innovation of this study lies in constructing a nomogram model integrating independent risk factors identified by multivariate analysis, which realizes non-invasive and accurate prediction of LNM in GC patients preoperatively. Therefore, this study aims to comprehensively evaluate the predictive value of immune, inflammatory, and tumour factors for LNM through a retrospective analysis of clinical data from GC patients, and to explore their correlation with clinical characteristics. The results are expected to provide a new theoretical basis for risk stratification and individualised treatment of GC patients, and lay the foundation for future multicentre prospective studies.

Materials and methods

Patient population

This research performed a retrospective analysis involving GC patients treated at Shidong Hospital, Yangpu District between March 2020 and March 2023, whose records were obtained from the hospital's electronic medical record system. Following rigorous inclusion and exclusion criteria, data from 207 patients were analyzed to construct a nomogram prediction model. Furthermore, patient data from April 2023 to March 2025 were gathered to create an external validation cohort for the clinical validation of the model. The study was reviewed and approved by the Institutional Review Board of Shidong Hospital, Yangpu District.

Sample size calculation

The sample size for the training cohort was estimated *a priori*. Based on previous studies reporting an LNM incidence of approximately 20-30% in clinically node-negative GC patients [6-8], and assuming an odds ratio (OR) of 2.0 for the primary predictors, a two-sided alpha of 0.05, and a power of 80%, the minimum required sample size was calculated to be at least 150-180 patients using established formulas for logistic regression (considering up to 8-10 candidate predictor variables). Our final training cohort of 207 patients exceeded this requirement, ensuring adequate statistical power for model development. The external validation cohort (n=97) was independently collected from a subsequent time period to assess model generalizability.

Inclusion and exclusion criteria

The inclusion criteria are as follows: (1) Age ≥18 years; (2) Patients diagnosed with GC through histopathologic examination and undergoing radical gastrectomy; (3) The preoperative examination was evaluated as stage N0; (4) Patients with complete clinical data, including baseline data and follow-up data.

The exclusion criteria are as follows: (1) Combined with other malignant tumors or autoimmune diseases; (2) Patients with severe dysfunction of vital organs such as the heart, liver and kidneys; (3) Patients who have undergone anticancer treatments such as radiotherapy, chemotherapy, and immunotherapy prior to surgery; (4) Patients who experience severe complications such as infection and bleeding during the perioperative period.

Determination of LNM

Postoperative LNM was determined based on histopathologic examination of resected lymph nodes. All lymph nodes dissected during surgery were formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin (H&E). Two experienced pathologists independently reviewed the slides to confirm the presence of metastatic cancer cells. Patients were divided into an LNM group (with histologically confirmed lymph node metastasis) and a non-LNM group (no lymph node metastasis detected in all resected nodes) based on this result.

Data collection

The patients' data was collected through the hospital's electronic medical record system. Baseline data: age, gender, body mass index (BMI), smoking history, drinking history, diabetes history, hypertension history, Helicobacter pylori (Hp) infection history, family history. Clinicopathological features: tumor size, tumor location, pathological type, degree of differentiation, depth of invasion, presence or absence of ulcers. Serological indicators: Carcinoembryonic Antigen (CEA), Carbohydrate Antigen 19-9 (CA199), Carbohydrate Antigen 72-4 (CA72-4), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Platelet-to-Lymphocyte Ratio (LMR), Interleukin-6 (IL-6), C-Reactive Protein (CRP) detected before the operation.

Statistical analysis

A statistical analysis was performed utilizing SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Data with a normal distribution were presented as mean ± standard deviation (SD), while the independent sample t-test was employed for comparisons between the two groups. The t-test was also used for the comparison of markers among subgroups with different clinical features. Categorical data were represented as n (%) and assessed using the chi-square or Fisher's exact test. The presence of collinearity was examined through the variance inflation factor (VIF), where a VIF of less than 5 indicates no collinearity. Variables showing a *P*-value of less than 0.10 by univariate logistic regression analysis were included in the multivariate logistic regression model to identify independent risk factors and develop a nomogram. The model's ability to discriminate was assessed by the area under the receiver operating characteristic (ROC) curve (AUC). The DeLong test was used to compare the differences among various AUC values. To evaluate the calibration, the Hosmer-Lemeshow test and calibration curve were used. The clinical benefits were measured through decision curve analysis (DCA). Internal validation was carried out using Bootstrap with 1,000 resampling iterations. An external validation cohort was formed by gathering patient data from various timeframes. A bilateral *P*-value of less than 0.05 was deemed significant.

Results

Comparison of general and clinical characteristics

A total of 207 patients were included in this study, among whom 55 (26.57%) had LNM (**Table 1**). In the LNM group, the proportions of patients with tumor diameters >3 cm (P<0.001), undifferentiated cancers (P=0.003), and T3-T4 stages (P=0.001) were significantly higher than those of the non-LNM group. No statistical differences in other characteristics were observed between the two groups of patients.

Comparison of immune inflammation and tumor indicators

The contents of CEA (9.56 vs. 7.12, P<0.001), CA199 (41.72 vs. 38.54, P=0.025) and CA72-4 (18.64 vs. 13.41, P<0.001) in the LNM group were significantly higher than those in the non-LNM group (**Table 2**). In terms of the immune-inflammation composite index, the NLR (2.41 vs. 1.82, P<0.001) and PLR (137.73 vs. 128.54, P=0.032) of patients in the LNM group were significantly higher than those of the non-LNM group, while the LMR (3.74 vs. 4.17, P=0.045) was significantly lower than that of the non-LNM group.

Association between clinical characteristics and various indicators

Based on the clinical characteristics of the patients as the grouping basis, the association between them and various serological indicators was explored. The results showed that patients with tumor diameters >3 cm exhibited higher levels of NLR (P<0.05) and IL-6 (P<0.01) (**Figure 1A**, **1B**). The levels of CA72-4 and IL-6 in patients at stage T3-T4 are significantly higher than those of patients at stage T1-T2 (P<0.05) (**Figure 1C**, **1D**).

Table 1. Comparison of baseline characteristics

Variable	Total (n=207)	Non-LNM group (n=152)	LNM group (n=55)	Statistic	*P*
Age, n (%)				χ^2=0.26	0.612
<60	85 (41.06)	64 (42.11)	21 (38.18)		
≥60	122 (58.94)	88 (57.89)	34 (61.82)		
Gender, n (%)				χ^2=0.24	0.622
Female	92 (44.44)	66 (43.42)	26 (47.27)		
Male	115 (55.56)	86 (56.58)	29 (52.73)		
BMI, Mean ± SD	22.92 ± 2.85	22.90 ± 2.73	22.97 ± 3.19	t=-0.17	0.868
Smoking, n (%)				χ^2=0.25	0.616
No	77 (37.20)	55 (36.18)	22 (40.00)		
Yes	130 (62.80)	97 (63.82)	33 (60.00)		
Drinking, n (%)				χ^2=2.52	0.112
No	98 (47.34)	77 (50.66)	21 (38.18)		
Yes	109 (52.66)	75 (49.34)	34 (61.82)		
Diabetes, n (%)				χ^2=1.00	0.318
No	164 (79.23)	123 (80.92)	41 (74.55)		
Yes	43 (20.77)	29 (19.08)	14 (25.45)		
Hypertension, n (%)				χ^2=0.42	0.519
No	128 (61.84)	92 (60.53)	36 (65.45)		
Yes	79 (38.16)	60 (39.47)	19 (34.55)		
Hp infection, n (%)				χ^2=1.46	0.227
No	106 (51.21)	74 (48.68)	32 (58.18)		
Yes	101 (48.79)	78 (51.32)	23 (41.82)		
Family history, n (%)				χ^2=0.01	0.917
No	176 (85.02)	129 (84.87)	47 (85.45)		
Yes	31 (14.98)	23 (15.13)	8 (14.55)		
Tumor size, n (%)				χ^2=15.05	<0.001
≤2 cm	121 (58.45)	101 (66.45)	20 (36.36)		
>3 cm	86 (41.55)	51 (33.55)	35 (63.64)		
Tumor site, n (%)				χ^2=5.97	0.051
Upper part	37 (17.87)	30 (19.74)	7 (12.73)		
Middle part	126 (60.87)	85 (55.92)	41 (74.55)		
Lower part	44 (21.26)	37 (24.34)	7 (12.73)		
Pathological pattern, n (%)				χ^2=2.94	0.087
Adenocarcinoma	147 (71.01)	103 (67.76)	44 (80.00)		
Non-adenocarcinoma	60 (28.99)	49 (32.24)	11 (20.00)		
Differentiated degree, n (%)				χ^2=9.07	0.003
Differentiated	129 (62.32)	104 (68.42)	25 (45.45)		
Undifferentiated	78 (37.68)	48 (31.58)	30 (54.55)		
Tumor infiltration, n (%)				χ^2=10.79	0.001
T1/T2	107 (51.69)	89 (58.55)	18 (32.73)		
T3/T4	100 (48.31)	63 (41.45)	37 (67.27)		
Ulceration, n (%)				χ^2=0.06	0.805
No	142 (68.60)	105 (69.08)	37 (67.27)		
Yes	65 (31.40)	47 (30.92)	18 (32.73)		

Note: LNM, lymph node metastasis; BMI, body mass index; SD, standard deviation; Hp, Helicobacter pylori.

Table 2. Comparison of immune inflammation and tumor indicators

Variable	Total (n=207)	Non-LNM group (n=152)	LNM group (n=55)	Statistic	*P*
CEA, Mean ± SD	7.76 ± 2.46	7.12 ± 2.15	9.56 ± 2.37	t=-7.00	<0.001
CA199, Mean ± SD	39.39 ± 7.48	38.54 ± 6.41	41.72 ± 9.54	t=-2.29	0.025
CA724, Mean ± SD	14.80 ± 4.99	13.41 ± 4.09	18.64 ± 5.23	t=-6.72	<0.001
NLR, Mean ± SD	1.98 ± 0.84	1.82 ± 0.77	2.41 ± 0.89	t=-4.60	<0.001
PLR, Mean ± SD	130.98 ± 24.49	128.54 ± 22.61	137.73 ± 28.17	t=-2.18	0.032
LMR, Mean ± SD	4.05 ± 1.33	4.17 ± 1.35	3.74 ± 1.22	t=2.02	0.045
IL6, Mean ± SD	8.25 ± 2.23	7.51 ± 1.77	10.29 ± 2.07	t=-9.51	<0.001
CRP, Mean ± SD	11.21 ± 2.61	10.91 ± 2.46	12.02 ± 2.87	t=-2.74	0.007

Note: LNM, lymph node metastasis; SD, standard deviation; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9; CA724, carbohydrate antigen 72-4; NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; LMR, Lymphocyte-to-Monocyte Ratio; IL6, Interleukin-6; CRP, C-Reactive Protein.

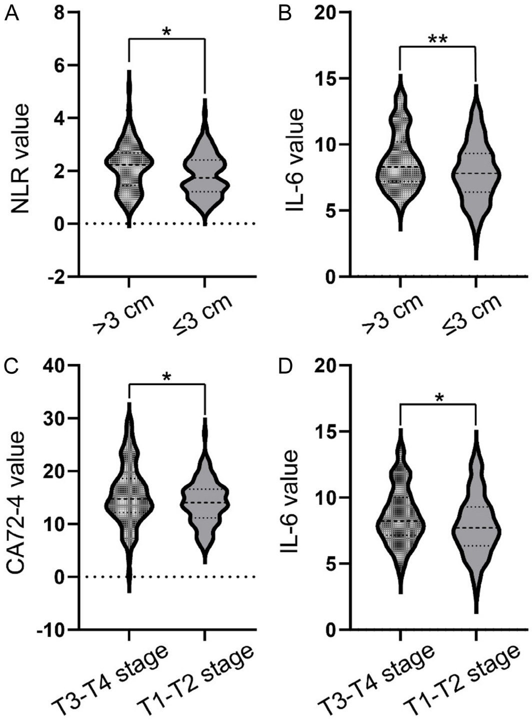

Figure 1. Association between clinical features and various indicators. A. Tumor size and NLR levels; B. Tumor size and IL-6 levels; C. Tumor infiltration and CA72-4 levels; D. Tumor infiltration and IL-6 levels. Note: NLR, Neutrophil-to-Lymphocyte Ratio; IL6, Interleukin-6; CA724, carbohydrate antigen 72-4. *P<0.05, **P<0.01.

Independent risk factors for LNM

Logistic regression analysis was used to explore the independent risk factors for LNM. The results showed that CEA (OR=1.52, 95% CI: 1.23-1.88, P<0.001), CA72-4 (OR=1.24, 95% CI: 1.11-1.38, P<0.001), NLR (OR=2.86, 95% CI: 1.58-5.17, P<0.001), and IL-6 (OR=1.97, 95% CI: 1.50-2.58, P<0.001) levels were independent risk factors for GC patients with LNM (**Table 3**). This indicates that for every 1 ng/L increase in serum IL-6 level, the odds of LNM increase by approximately 97%. The VIF values of these four variables are all less than 5, indicating a lack of a collinearity problem. The nomogram prediction model constructed based on multivariate Logistic regression is shown in **Figure 2**. This nomogram integrates four independent risk factors and predicts the probability of patients developing LNM by calculating the total risk score.

Evaluation of the predictive model

The ROC curve is shown in **Figure 3A**, indicating that this prediction model had good discrimination for patients with LNM (AUC=0.93). The calibration curve is shown in **Figure 3B**. The results show that there is no significant difference between the observed values and the predicted values, and the model fits well (Hosmer-Lemeshow P=0.63). The DCA results show that when the risk threshold is between 0.1 and 1.0, the intervention based on this model can bring positive net benefits (**Figure 3C**).

Internal validation of the prediction model

Internal validation was conducted by repeated sampling 1,000 times using the Bootstrap method. The ROC curve of the internal validation queue is shown in **Figure 4A**, which demonstrates good discrimination (AUC=0.92). The calibration curve indicates that the model fits well (Hosmer-Lemeshow P=0.34) (**Figure 4B**). The DCA results show that this model has excellent clinical application value (**Figure 4C**).

Table 3. Multivariate Logistic regression analysis

Variable	β	SE	Z	*P*	OR (95% CI)	VIF
CEA	0.42	0.11	3.83	<0.001	1.52 (1.23-1.88)	1.15
CA724	0.21	0.06	3.75	<0.001	1.24 (1.11-1.38)	1.16
NLR	1.05	0.30	3.47	<0.001	2.86 (1.58-5.17)	1.06
IL6	0.68	0.14	4.92	<0.001	1.97 (1.50-2.58)	1.17

Note: SE, standard error; OR, odds ratio; CI, confidence interval; VIF, Variance inflation factor; CEA, carcinoembryonic antigen; CA724, carbohydrate antigen 72-4; NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; IL6, Interleukin-6.

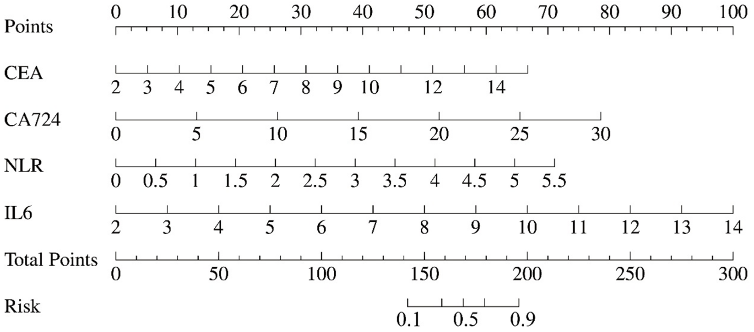

Figure 2. Nomogram prediction model. Note: CEA, carcinoembryonic antigen; NLR, Neutrophil-to-Lymphocyte Ratio; IL6, Interleukin-6; CA724, carbohydrate antigen 72-4.

External validation of the prediction model

A total of 97 GC patients were included in the external validation cohort, among which 24 (24.74%) cases were combined with LNM. The comparison of baseline characteristics between the external validation queue and the original modeling queue is shown in **Table 4**. There were no significant differences in basic and clinical characteristics between the patients in the two cohorts. The ROC curve (AUC=0.91), calibration curve (Hosmer-Lemeshow P=0.74) and DCA of the external validation cohort all indicated that the model had good predictive efficacy (**Figure 4D-F**).

Comparative analysis with traditional clinicopathologic features

To further validate the clinical utility of our nomogram model, we compared its predictive performance for LNM with that of three conventional clinicopathologic features-tumor size (>3 cm vs. ≤3 cm), tumor differentiation (undifferentiated vs. differentiated), and depth of tumor infiltration (T3-T4 vs. T1-T2). ROC analyses were performed for each feature, and the AUC was calculated. The AUC values for tumor size, differentiation degree, and tumor infiltration were 0.65 (95% CI: 0.57-0.74), 0.62 (95% CI: 0.53-0.70), and 0.63 (95% CI: 0.54-0.71), respectively (**Table 5**). All three features exhibited significantly lower discriminatory power compared to our nomogram model (AUC=0.93, P<0.001 for each comparison, DeLong's test). These results underscore the superior predictive ability of the integrated nomogram over individual conventional pathological indicators in identifying GC patients at high risk of LNM.

Discussion

Gastric carcinoma (GC) is a malignant tumor of the digestive tract with a high incidence and mortality rate worldwide [17]. Postoperative LNM is a key factor affecting the prognosis of patients. This study retrospectively analyzed the clinical data of 207 patients with GC and systematically evaluated the correlations between immune inflammatory indicators (such as NLR, PLR, LMR, IL-6, and CRP) and tumor markers (CEA, CA199, CA72-4) and LNM as well as pathologic features. A Nomogram prediction model based on multivariate Logistic regression was constructed. The results showed that CEA, CA72-4, NLR, and IL-6 were independent risk factors for LNM, and the model demonstrated good predictive performance in both internal and external validation. These findings provide a new theoretical basis for postoperative risk stratification and individual treatment in gastric cancer patients.

This study found that NLR and PLR were significantly higher in the LNM group than in the non-LNM group, while LMR was significantly lower, consistent with multiple previous studies. Kotecha et al. demonstrated through a meta-analysis of 12 studies (n=3892) that elevated NLR was associated with a 1.90-fold increased risk of LNM in GC, which aligns with our finding that NLR is an independent risk factor [18]. Zhang et al. reported in a large cohort (n=904)

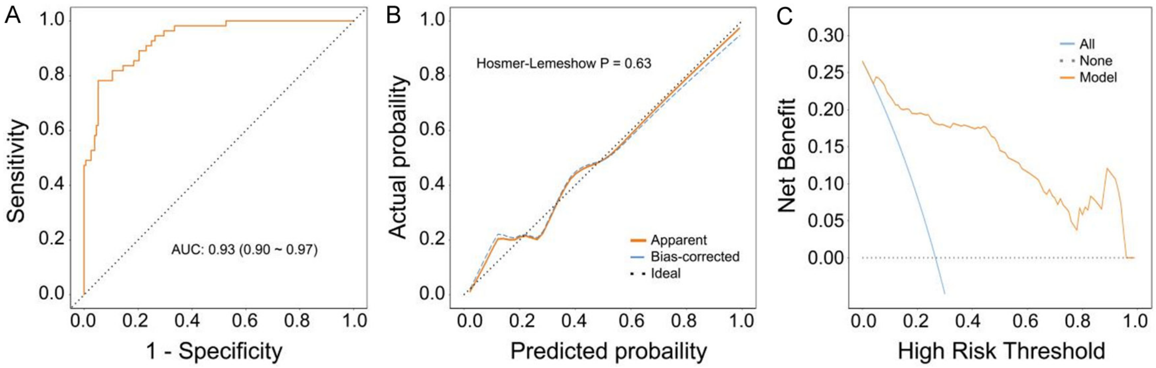

Figure 3. Evaluation of the predictive model. A. ROC curve; B. Calibration curve analysis; C. DCA curve. Note: ROC, receiver operating characteristic; AUC, area under the curve; DCA, decision curve analysis.

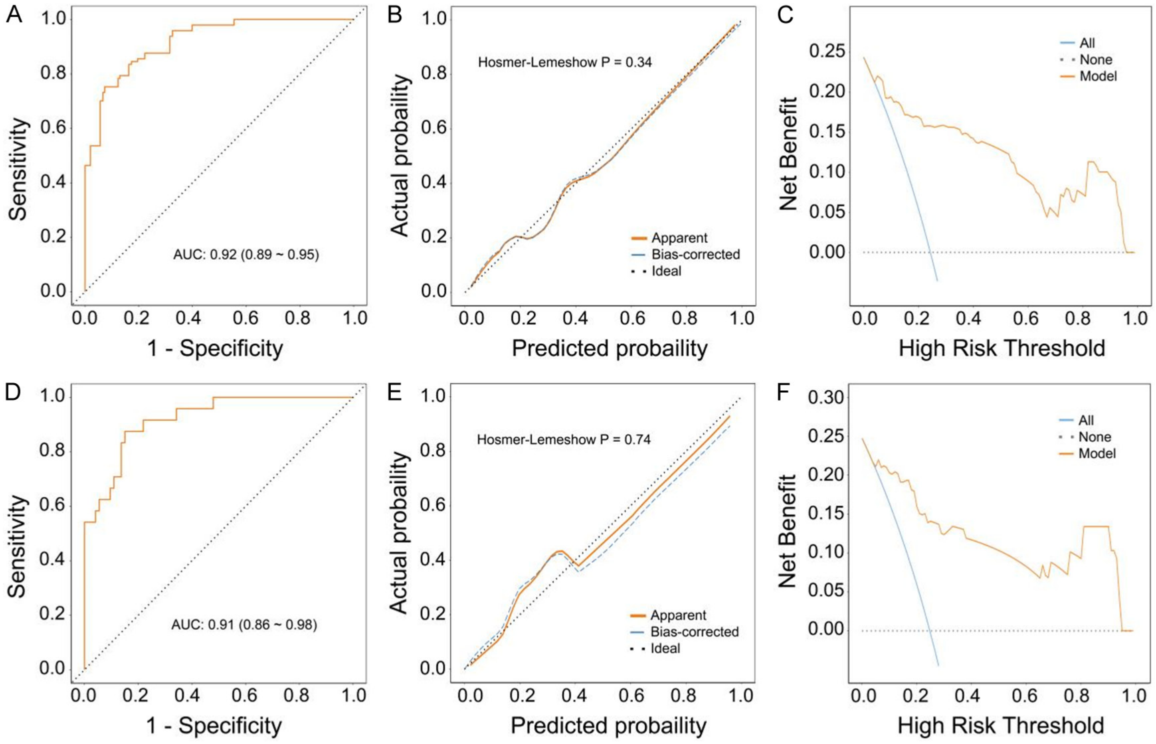

Figure 4. Evaluation of the predictive model of the verification queue. A. ROC curve of the internal validation cohort; B. Calibration curve of the internal validation cohort; C. DCA curve of the internal validation cohort; D. ROC curve of the external validation cohort; E. Calibration curve of the external validation cohort; F. DCA curve of the external validation cohort. Note: ROC, receiver operating characteristic; AUC, area under the curve; DCA, decision curve analysis.

that both NLR and PLR were positively correlated with LNM, but their study did not identify these ratios as independent predictors, possibly due to differences in patient baseline characteristics (e.g., higher proportion of advanced T-stage patients) [19]. Ma et al. further confirmed that low LMR was significantly associated with LNM and poor prognosis in GC, and proposed that the imbalance between lymphocytes and monocytes reflects the suppression of anti-tumor immunity [20]. Compared to these studies, our work integrates NLR with IL-6, CEA, and CA72-4 to construct a predictive model, which enhances the clinical applicability of combining systemic inflammatory markers with tumor-specific indicators. The underlying mechanism may involve the crosstalk between systemic inflammation and tumor progression.

Table 4. Feature comparison between the modeling group and the external group

Variable	Total (n=304)	Modeling group (n=207)	External group (n=97)	Statistic	*P*
Age, n (%)				χ^2=0.43	0.512
<60	121 (39.80)	85 (41.06)	36 (37.11)		
≥60	183 (60.20)	122 (58.94)	61 (62.89)		
Gender, n (%)				χ^2=0.02	0.881
Female	136 (44.74)	92 (44.44)	44 (45.36)		
Male	168 (55.26)	115 (55.56)	53 (54.64)		
BMI, Mean ± SD	22.83 ± 2.90	22.92 ± 2.85	22.65 ± 2.99	t=0.74	0.458
Smoking, n (%)				χ^2=0.03	0.874
No	114 (37.50)	77 (37.20)	37 (38.14)		
Yes	190 (62.50)	130 (62.80)	60 (61.86)		
Drinking, n (%)				χ^2=1.36	0.244
No	137 (45.07)	98 (47.34)	39 (40.21)		
Yes	167 (54.93)	109 (52.66)	58 (59.79)		
Diabetes, n (%)				χ^2=0.77	0.379
No	245 (80.59)	164 (79.23)	81 (83.51)		
Yes	59 (19.41)	43 (20.77)	16 (16.49)		
Hypertension, n (%)				χ^2=0.03	0.860
No	189 (62.17)	128 (61.84)	61 (62.89)		
Yes	115 (37.83)	79 (38.16)	36 (37.11)		
Hp infection, n (%)				χ^2=0.61	0.434
No	151 (49.67)	106 (51.21)	45 (46.39)		
Yes	153 (50.33)	101 (48.79)	52 (53.61)		
Family history, n (%)				χ^2=0.37	0.544
No	261 (85.86)	176 (85.02)	85 (87.63)		
Yes	43 (14.14)	31 (14.98)	12 (12.37)		
Tumor size, n (%)				χ^2=0.08	0.773
≤2 cm	176 (57.89)	121 (58.45)	55 (56.70)		
>3 cm	128 (42.11)	86 (41.55)	42 (43.30)		
Tumor site, n (%)				χ^2=0.13	0.935
Upper part	53 (17.43)	37 (17.87)	16 (16.49)		
Middle part	185 (60.86)	126 (60.87)	59 (60.82)		
Lower part	66 (21.71)	44 (21.26)	22 (22.68)		
Pathological pattern, n (%)				χ^2=1.55	0.213
Adenocarcinoma	209 (68.75)	147 (71.01)	62 (63.92)		
Non-adenocarcinoma	95 (31.25)	60 (28.99)	35 (36.08)		
Differentiated degree, n (%)				χ^2=0.01	0.924
Differentiated	190 (62.50)	129 (62.32)	61 (62.89)		
Undifferentiated	114 (37.50)	78 (37.68)	36 (37.11)		
Tumor infiltration, n (%)				χ^2=0.00	0.981
T1/T2	157 (51.64)	107 (51.69)	50 (51.55)		
T3/T4	147 (48.36)	100 (48.31)	47 (48.45)		
Ulceration, n (%)				χ^2=0.08	0.782
No	207 (68.09)	142 (68.60)	65 (67.01)		
Yes	97 (31.91)	65 (31.40)	32 (32.99)		

Note: LNM, lymph node metastasis; BMI, body mass index; SD, standard deviation; Hp, Helicobacter pylori.

Table 5. Comparison of predictive performance between the nomogram model and traditional clinicopathologic features

Indicator	AUC	95% CI	AUC difference	DeLong's Test	
				Z value	*P* value
Tumor size	0.65	0.57-0.74	-0.28	-6.75	<0.001
Differentiated degree	0.62	0.53-0.70	-0.32	-7.45	<0.001
Tumor infiltration	0.63	0.54-0.71	-0.31	-7.48	<0.001

Note: AUC, area under the curve; CI, confidence interval.

Existing evidence indicates that neutrophils can destroy the extracellular matrix by secreting matrix metalloproteinases (MMPs) and reactive oxygen species (ROS), creating conditions for tumor cell invasion [21, 22]. In addition, neutrophil-derived inflammatory factors can further activate signaling pathways such as NF-κB, promoting the survival and migration of tumor cells [23]. The reduction of the LMR may reflect the impairment of the anti-tumor immune response mediated by lymphocytes [24]. Shen et al. also reported through retrospective analysis that a lower LMR was associated with a poorer survival prognosis in GC patients [25]. It is worth noting that IL-6 was identified as an independent risk factor for LNM in this study. Multivariate analysis revealed that each 1 ng/L increase in serum IL-6 was associated with a 97% increase in the odds of LNM (OR=1.97). Our finding that elevated serum IL-6 predicts LNM aligns with and extends the mechanistic insights from prior experimental work. For instance, Liu et al. demonstrated in gastric cancer cells that IL-6 activates the JAK/STAT3 pathway to promote proliferation and invasion [26], while Xu et al. reported that IL-6 can induce VEGF expression [27]. In the context of these studies, our clinical data suggest that the systemic IL-6 elevation detected in patients may be driving these same pro-metastatic pathways *in vivo*, thereby creating a microenvironment conducive to lymphatic spread. This positions IL-6 not merely as an inflammatory marker but as a potential mediator linking systemic inflammation to local metastatic progression.

In this study, elevated preoperative serum levels of CEA and CA72-4 were independently associated with a higher risk of LNM. This corroborates the findings of Zhang et al., who in a larger cohort (n=1,667) also reported CEA and CA72-4 as significant predictors of gastric cancer metastasis [28]. However, our study specifically validates their predictive value for LNM in a clinically node-negative preoperative setting, which is crucial for surgical planning. Regarding potential mechanisms, Wu et al. reviewed that CEA can promote lymphatic vessel infiltration by interaction with CEACAM6 [29], and Chen et al. suggested that CEA might confer chemoresistance through the PI3K/AKT pathway [30]. CA72-4 is a more specific biomarker for gastric cancer, and its high expression is closely related to the poor prognosis of gastric cancer [31]. Similarly, for CA72-4, while Xu et al. hypothesized that its high expression might enhance tumor cell adhesion and detachment, our clinical association supports the notion that this glycoprotein plays a role in the metastatic cascade, possibly at the stage of lymphatic vessel entry [32]. Of course, its specific mechanism requires further experimental research to verify. It is worth noting that although CA199 showed significant differences in the univariate analysis, it did not enter the final model, which may be related to the fact that CA199 is more specific to peritoneal metastasis, suggesting that different tumour markers have 'site preferences' in the metastasis pathway [33]. This study also found that tumour diameter >3 cm, low differentiation, and T3-T4 stage were significantly associated with LNM, which is consistent with the biological behavior of tumours: larger tumors are more likely to break through the basement membrane, poorly differentiated tumour cells are more invasive, and T3-T4 stage directly reflects the depth of tumour invasion and the risk of lymphatic vessel invasion [34].

In this study, the predictive model constructed based on CEA, CA724, NLR, and IL6 demonstrated excellent predictive performance in the modelling cohort, internal validation, and external validation. Calibration curve and decision curve analysis confirmed its clinical utility. Compared with traditional pathologic staging, this model can achieve non-invasive assessment through preoperative serological testing, which is helpful for identifying patients with high LNM risk before surgery and provides a basis for the selection of lymph node dissection range and the decision-making of adjuvant treatment timing. Nevertheless, this study still has some limitations. First, this study was a single-centre retrospective study with a relatively limited sample size, which may have introduced selection bias. Future studies should be conducted as multicentre prospective studies to validate the findings of this study and enhance their generalizability. Second, due to the retrospective nature of the study, we were unable to perform immunohistochemistry or molecular biology experiments to directly validate the expression levels of key markers such as CEA, CA72-4, and IL-6 in gastric cancer tissues from LNM-positive and LNM-negative patients. While the serological associations reported here are supported by previous literature and have demonstrated strong predictive value in our model, direct tissue-based evidence would further strengthen the biological plausibility of our findings. Future prospective studies incorporating tissue biopsies are warranted to elucidate the local tumor microenvironmental role of these markers. Third, although this study identified IL-6 as an independent risk factor for LNM in GC patients, the specific molecular mechanism by which IL-6 regulates LNM remains unclear. Future *in vitro* and *in vivo* studies - such as evaluating the impact of IL-6 on gastric cancer cell migration, invasion, and lymphangiogenesis in cell culture and animal models - are warranted to uncover the precise biological role of IL-6 and other identified markers. Such mechanistic investigations would strengthen the translational medicine value of our findings and may reveal novel therapeutic targets for inhibiting LNM. Additionally, while the predictive model developed in this study demonstrated good predictive performance, its clinical utility requires confirmation through large-scale clinical validation. Future efforts could integrate artificial intelligence and machine learning technologies to further optimise the predictive model and enhance its predictive accuracy and practicality.

Conclusion

This study thoroughly investigated the association between LNM in GC patients after surgery and immune inflammatory markers and tumour markers. Through a retrospective analysis of clinical data from 207 GC patients, we found that CEA, CA72-4, NLR, and IL-6 levels were independent risk factors for LNM. The predictive model constructed based on these factors demonstrated good predictive performance, providing a new theoretical basis for risk stratification and personalized treatment in GC patients. This study not only reveals the important role of immune inflammatory responses and tumour markers in GC progression but also highlights their potential application value for predicting LNM. In the future, through multicentre prospective studies and in-depth mechanistic investigations, we will further optimize the predictive model to enhance its clinical application value.

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Disclosure of conflict of interest

None.

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