# Prognostic role and clinical association of neutrophil/lymphocyte ratio with immune infiltrates in gastric cancer

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# Abstract

**Background:** Neutrophil/lymphocyte ratio (NLR) is a vital index for systemic inflammation and prognostic indicator for gastric cancer (GC). More details between NLR and prognosis were still obscure. The objective of this study was to analyze the role of NLR in different prognostic models and subgroups, and explore the mediating effects of immune infiltrates between NLR and survival.

**Methods:** A total of 924 patients underwent D2 radical gastrectomy were enrolled in this study. According to the level of NLR, patients were divided into two groups, the high and low NLR groups. Clinical parameters, indexes related to immune infiltrates, and survival were compared between the two groups. Prognostic models, interaction analysis and mediating effects analysis were performed to investigate the clinical association of NLR, immune infiltrates, and survival.

**Results:** The infiltration of CD3+ and CD8+ T cells was significantly different in the two NLR groups. The level of NLR was an independent prognostic predictor of GC. The NLR-adjusted pTNM staging models had better predictive value for patient prognosis when compared with the traditional pTNM staging system. In addition, an interaction effect exists between NLR and MMR status on the prognosis of GC ($P_{interaction} < 0.01$). High level of NLR correlated with a better prognosis in patients with dMMR, while associated with a worse prognosis in patients with pMMR. Lastly, the mediating effect analysis revealed that the infiltration level of CD3+ T cells was the mediating factor between NLR and survival ($P < 0.001$).

**Conclusions:** The level of NLR is an independent prognostic predictor of GC. Adding an index representing the level of NLR can improve the prognostic prediction value of TNM staging system in GC patients. The effect of NLR on prognosis is partly mediated by CD3+ T cells infiltration.


# Background

Gastric cancer is a worldwide health problem. More than one million new cases of gastric cancer are diagnosed in the world each year [1]. Radical surgery with D2 dissection is the only cure for gastric cancer. With the development of chemotherapy, the prognosis of gastric cancer patients has been improved while still not satisfactory.

About 10% of lymph node negative gastric cancer patients who underwent curative surgery would suffer from cancer recurrence [2, 3]. The TNM staging system is commonly used to predict cancer patients' prognosis and guide postoperative treatment. However, many prognostic factors were not included in this system. Many other indexes, such as tumor-infiltrating lymphocytes (TIL) and markers of systemic inflammation, were reported as prognostic factors [4, 5]. Therefore, there is an urgent need to find new prognostic markers to optimize the TNM staging system to predict patient prognosis better.

The tumor immune microenvironment is essential for tumor initiation and progression. Studies indicated that markers of inflammation were independent prognostic predictors for patient survival with multiple malignancies. The neutrophil-to-lymphocyte ratio (NLR) is one of the systemic inflammation markers

correlated with clinical outcomes of various cancers, including gastric cancer, colorectal cancer, pancreatic cancer, and liver cancer [6–10]. As many inflammatory cells are known to migrate from peripheral blood to local tissues via systemic circulation, NLR is found associated with the density of CD4 + immune cells or the presence of tertiary lymphoid structures (TLS), which leads to its prognostic values in gastric cancer [11, 12]. Nevertheless, evidence is still lacking to explain how much a role TIL plays in the poor prognosis of NLR. Immune checkpoint besides TLS is a significant selecting index for patients who will most likely benefit from cancer immunotherapy. More studies are needed to evaluate the relationship between NLR and immune infiltrates or immune checkpoints, and their roles in prognosis prediction of gastric cancer.

Therefore, in the present study, we systematically evaluated the prognostic value of NLR, accessed the prognosis of NLR in different immune checkpoints subgroups, and investigated the mediation effect between NLR and survival mediated through lymphocytic infiltrates in gastric cancer.

## Methods

# Patient selection

We conducted a retrospective study in eligible GC patients who underwent gastrectomy at Peking University Cancer Hospital between June 2003 and December 2012. The patients were selected from Xiaofang Xing's previous research studying T cell infiltration (TCI) in GC [13]. The inclusion criteria of the patient including patients with (1) available FFPE tissues; (2) histologic identification of the adenocarcinoma; (3) without preoperative chemotherapy or radiotherapy. The exclusion criteria were the patients (1) without blood index information, such as neutrophil counts and lymphocyte counts, within two weeks before the operation; (2) With preoperative sepsis or confirmed systemic infection. The PUCH Ethics Committee approved the study, and all participants included in the present study have provided informed consent. Follow-up data were retrieved from hospital records.

# Definition of neutrophil-to-lymphocyte ratio

We used the neutrophil-to-lymphocyte ratio (NLR) as the marker of systemic inflammation in this study, which was calculated as the neutrophil count ratio to the lymphocyte count. An optimal cutoff value was defined to classify the samples into two groups (high NLR vs. low NLR) for the value of NLR using X-tile plots based on the association with the patients' overall survival. The X-tile plots can automatically generate the optimum cutoff point for continuous variables according to the highest $\chi^2$ value (minimum *p*-value) defined by Kaplan–Meier survival analysis and log-rank test. The cutoff value for the NLR was determined to be 3.09 in our study, with the low NLR (< 3.09) group showing a significantly higher survival rate than that with high NLR (> 3.09) in Kaplan-Meier analysis ($P < 0.001$, Fig. 1).

# Immunohistochemical staining

We built tissue microarrays (TMAs) from surgical specimens preserved in paraffin blocks. Then, we performed immunohistochemistry on the section of TMAs after deparaffinization. Arrays were stained

with the following primary antibodies: antiCD3 antibody (NCL-CD3-565, Leica), anti-CD8 antibody (CRM311C, Biocare Medical), anti-MutL homolog 1 (MLH1), antibody (IR079, DAKO), anti-mutS homolog 2 (MSH2) antibody (IR085, DAKO), anti-mutS homolog 6 (MSH6) antibody (IR086, DAKO), and anti-PD-L1 antibody (SP142, Roche). Details of the protocols and scoring schema for the hMLH1, MSH2, MSH6, PDL1, CD3, and CD8 were presented in the previous study of Xiaofang Xing, et al.

# Evaluation of immunohistochemistry staining

The densities of CD3 + and CD8 + immune cells infiltration were assessed with the percentage immunoreactivity (positive cells/ (positive cells + negative cells)*100)), which was used as a surrogate for the extent of immune cell infiltration. A previously described image analysis system was used to identify all strained cores of immune cells and was performed as follows. Stained TMAs slides were scanned at x20 magnification with an Aperio XT digital slide scanner and subjected to automatic image analysis to identify and quantify immunoreactivity. Brown (immunopositive) pixels, blue (immunonegative) pixels and white (empty space) pixels in slides were discriminated against by the TMAs system, an in-house developed software. A senior GI pathologist reviewed all cores after the image analysis process and confirmed that (a) the detection of the brown staining had been performed accurately by the software and (b) to exclude all cores which contained tumor cells.

The expression of MMR genes was briefly defined as follows. MMR-proficient (pMMR) status referred to simultaneously expressing MLH1, MSH2 and MSH6, while MMR-deficient (dMMR) states were defined as the loss of MLH1 and loss of both MSH6 and MSH2 expression. The immunostaining of PD-L1 in tumors was rated as presence or absence. The consensus among three pathologists determined the final interpretation. A high level of consistency and few discrepant cases (< 5%) was reached after joint review.

# Statistical analysis

We computed the Kaplan–Meier curves and log-rank test based on X-tile software to compare OS between low and high NLR groups. Distributions of continuous variables between two groups were described as mean or median, and standard deviation or interquartile range and compared using the t-test or, in cases of non-normality, Mann-Whitney test. Categorical variables were described as numbers with percentages and compared using the $\chi 2$ test or Fisher's exact test. We performed univariate and multivariate cox proportional hazard regression models to calculate the hazard ratios (HR). In the Cox regression models, we considered the covariables associated with NLR level, survival outcome, and tumor microenvironment, including age (< 65 vs. >=65), gender (female vs. male), pTNM stage (I vs. II vs. III vs. IV), site (EGJ vs. GC), Lauren type (diffuse vs. Intestinal vs. mixed), differentiation grade (poorly or not), MMR status (proficient vs. Deficient), PDL1 expression (positive vs. negative), and the densities of CD3 + and CD8 + immune cells.

Moreover, we used mediation analysis to explore the role of TCI that explained the NLR disparities in the survival rate among GC patients. We further performed subgroup analyses to investigate the HR of different NLR levels across different subgroups stratified by age, gender, tumor site, pTNM stage, differentiation grade, Lauren type, MMR status, and PDL1 expression. We also conducted an interaction

test to evaluate the heterogeneity of treatment effect across the subgroups. Besides, we evaluated the clinical value of the NLR for survival prediction by comparing the Cox regression model with or without adjustment of NLR. The predictive values for survival were determined by Harrell's C-statistic (C-index). The goodness of fit was determined by the Akaike Information Criterion (AIC). The receiver operating characteristic (ROC) curve and decision curve analysis (DCA) were employed to evaluate the performance and net benefit of the NLR-adjusted model in a clinical context.

We performed the X-tile plots with the X-tile software version 3.6.1 (Yale University School of Medicine, New Haven, CT, USA). We conducted all the other statistical tests with R software version 4.0.1 (with "mediation" package for mediation analysis, "survivalROC" package and "timeROC" package for ROC curve, "Stats" package for AIC, "survival" package for Cox regression and C-index, "rmda" package for DCA, and "ggplot2" and "forestplot" for plot figures) (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was declaimed with two-sided $P < 0.05$ for all tests.

## Results

# Patient characteristics

A total of 924 patients with resected FFPE tissue and preoperative blood indexes were included in our study. Table 1 summarizes the patient-related, tumor-related, and microenvironment-related characteristics. The mean age was 60.16 years (range 22–89 years). Among all patients, female patients accounted for 28.0%, and those aged 65 years or older made up 36.9%. Most tumors were located at the gastric part (76.3%) and were identified as an intestinal-type (55.3%) or diffuse type (24.7%) by the Lauren classification. Approximately half of the patients (46.4%) exhibited poor differentiation. MMR-deficient tumor and PDL1 positive tumor were observed in 91 patients (9.8%) and 351 patients (38.0%) respectively. The percentages of cancers by stage were as follows: 11.8% for stage I cancer, 27.8% for stage II cancer, 52.8% for stage III cancer, and 7.6% for stage IV cancer. The mean density of CD3 + and CD8 + T cells were 12.79% and 11.03%, respectively.

Table 1
Clinicopathological and molecular features according to NLR level.

| Variable | N | Overall, N = 924[a)] | Low NLR, N = 682[a)] | High LNR, N = 242[a)] | *P*-value[b)] |
|---|---|---|---|---|---|
| Age | 924 | | | | < 0.001 |
| <65 | | 583 (63.1%) | 453 (66.4%) | 130 (53.7%) | |
| >=65 | | 341 (36.9%) | 229 (33.6%) | 112 (46.3%) | |
| Gender | 924 | | | | 0.637 |
| female | | 259 (28.0%) | 194 (28.4%) | 65 (26.9%) | |
| male | | 665 (72.0%) | 488 (71.6%) | 177 (73.1%) | |
| pTNM | 924 | | | | 0.004 |
|  | | 109 (11.8%) | 87 (12.8%) | 22 (9.1%) | |
|  | | 257 (27.8%) | 198 (29.0%) | 59 (24.4%) | |
|  | | 488 (52.8%) | 357 (52.3%) | 131 (54.1%) | |
|  | | 70 (7.6%) | 40 (5.9%) | 30 (12.4%) | |
| Site | 924 | | | | 0.100 |
| EGJ | | 219 (23.7%) | 171 (25.1%) | 48 (19.8%) | |
| GC | | 705 (76.3%) | 511 (74.9%) | 194 (80.2%) | |
| Lauren type | 924 | | | | 0.379 |
| diffuse | | 228 (24.7%) | 170 (24.9%) | 58 (24.0%) | |
| intestinal | | 511 (55.3%) | 367 (53.8%) | 144 (59.5%) | |
| mixed | | 168 (18.2%) | 131 (19.2%) | 37 (15.3%) | |
| (missing) | | 17 (1.8%) | 14 (2.1%) | 3 (1.2%) | |
| Differentiation | 924 | | | | 0.572 |
| poorly | | 429 (46.4%) | 313 (45.9%) | 116 (47.9%) | |
| moderately or well | | 450 (48.7%) | 338 (49.6%) | 112 (46.3%) | |
| (missing) | | 45 (4.9%) | 31 (4.5%) | 14 (5.8%) | |

[a)] Mean (SD); n (%)

[b)] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

| Variable | N | Overall, N = 924[a)] | Low NLR, N = 682[a)] | High LNR, N = 242[a)] | *P*-value[b)] |
|---|---|---|---|---|---|
| MMR | 924 | | | | 0.642 |
| proficient | | 706 (76.4%) | 518 (76.0%) | 188 (77.7%) | |
| deficient | | 91 (9.8%) | 66 (9.7%) | 25 (10.3%) | |
| (missing) | | 127 (13.7%) | 98 (14.4%) | 29 (12.0%) | |
| CD3 | 924 | 12.79% (9.02) | 13.33% (9.21) | 11.28% (8.30) | < 0.001 |
| CD8 | 924 | 11.03% (7.77) | 11.48% (8.27) | 9.75% (5.96) | 0.022 |
| PDL1 | 924 | | | | 0.285 |
| negative | | 573 (62.0%) | 416 (61.0%) | 157 (64.9%) | |
| positive | | 351 (38.0%) | 266 (39.0%) | 85 (35.1%) | |

[a)] Mean (SD); n (%)

[b)] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Using the defined cutoff value of NLR (3.09), we identified 682 patients (73.8%) as having a low NLR and 242 patients (26.2%) as having a high NLR. The high NLR group displayed a higher proportion of patients with old age ($P < 0.001$) and late pTNM stage ($P = 0.004$), relative to the low NLR group. High density of CD3+ ($P < 0.001$) and CD8+ ($P = 0.022$) immune cells infiltration was also observed in the low NLR group. The NLR was not correlated with gender, tumor site, lauren type, histologic differentiation degree, MMR status or PDL1 expression.

# NLR and survival outcomes

In the univariable Cox regression analysis, high NLR, old age, and high pTNM stage have significantly increased the risk of mortality (Fig. 1 and Table 2). GC patients with a high density of TCI, Lauren intestinal type, and PDL1 expression in tumors were showed better survival (Table 2). Multivariate regression analysis indicated NLR, age stratification, pTNM stage, Lauren type, CD3 + TCI, and PDL1 expression in a tumor significantly associated with OS after controlling for the major confounders (Table 2).

Table 2
Univariate and multivariate analysis of overall survival (OS) rates.

| Characteristic | Univariable | | | Multivariable | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P*-value | HR | 95% CI | *P*- value |
| NLR | 1.74 | 1.43, 2.12 | < 0.001 | 1.58 | 1.29, 1.93 | < 0.001 |
| Age | | | | | | |
| <65 | — | — | | — | — | |
| >=65 | 1.49 | 1.24, 1.80 | < 0.001 | 1.64 | 1.35, 2.00 | < 0.001 |
| Gender | | | | | | |
| female | — | — | | — | — | |
| male | 1.13 | 0.92, 1.40 | 0.244 | 1.09 | 0.88, 1.36 | 0.425 |
| pTNM | | | | | | |
|  | — | — | | — | — | |
|  | 3.59 | 1.91, 6.73 | < 0.001 | 3.34 | 1.78, 6.27 | < 0.001 |
|  | 9.13 | 5.00, 16.68 | < 0.001 | 8.48 | 4.62, 15.5 | < 0.001 |
|  | 24.16 | 12.65, 46.14 | < 0.001 | 23.1 | 12.0, 44.4 | < 0.001 |
| Site | | | | | | |
| EGJ | — | — | | — | — | |
| GC | 0.99 | 0.79, 1.23 | 0.908 | 0.85 | 0.68, 1.06 | 0.139 |
| Differentiation | | | | | | |
| poorly | — | — | | — | — | |
| moderately or well | 0.84 | 0.69, 1.01 | 0.062 | 1.00 | 0.80, 1.25 | 0.999 |
| (missing) | 0.7 | 0.43, 1.15 | 0.164 | 0.60 | 0.36, 1.00 | 0.051 |
| Lauren type | | | | | | |
| diffuse | — | — | | — | — | |
| intestinal | 0.75 | 0.6, 0.93 | 0.008 | 0.66 | 0.51, 0.85 | 0.002 |
| mixed | 0.9 | 0.69, 1.18 | 0.449 | 0.87 | 0.65, 1.16 | 0.334 |
| (missing) | 0.39 | 0.14, 1.05 | 0.062 | 0.62 | 0.22, 1.70 | 0.349 |

[a)] HR: Hazard ratio; CI: Confidence interval

| Characteristic | Univariable | | | Multivariable | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P*-value | HR | 95% CI | *P*- value |
| MMR | | | | | | |
| proficient | — | — | | — | — | |
| deficient | 0.81 | 0.58, 1.13 | 0.206 | 0.85 | 0.60, 1.20 | 0.362 |
| (missing) | 0.91 | 0.69, 1.21 | 0.527 | 0.80 | 0.60, 1.06 | 0.120 |
| CD3 | 0.97 | 0.96, 0.98 | < 0.001 | 0.98 | 0.97, 0.99 | 0.004 |
| CD8 | 0.97 | 0.96, 0.99 | < 0.001 | 1.00 | 0.99, 1.02 | 0.750 |
| PDL1 | | | | | | |
| negative | — | — | | — | — | |
| positive | 0.69 | 0.57, 0.85 | < 0.001 | 0.78 | 0.64, 0.96 | 0.021 |
| [a)] HR: Hazard ratio; CI: Confidence interval | | | | | | |

# Subgroup analysis

We performed a subgroup analysis of the effect of NLR (high group *vs* low group) according to age, gender, tumor site, pTNM stage, differentiation grade, Lauren type, MMR status and PDL1 expression (Fig. 2). High NLR was associated with a worse prognosis across age, gender, tumor site, pTNM stage, differentiation grade, lauren type, and PDL1 expression due to the values of HR greater than 1. In addition, subgroup analysis according to MMR status demonstrated high NLR improve OS among patients with dMMR (HR, 0.69; 95% CI, 0.28–1.66), but not in those with pMMR (HR, 1.77; 95% CI, 1.41–2.23), (*P*-interaction < 0.01), (Fig. 2 and Supplementary Fig. 1).

# Prognostic value of NLR

The multivariable regression and subgroup analyses indicated that NLR stratification was a prognostic predictor independent from the pTNM staging system (Table 2 and Fig. 3A). Thus, we further developed two models with or without NLR to assess the predictive value of the NLR. The model 1 was TNM multivariable Cox model based on the pTNM stage, age, CD3 + T cell infiltration, Lauren classification and PDL1 expression, which were identified as the independent prognostic factors of gastric cancer. And the other model 2 was TNM univariable Cox model. (Table 3)

Table 3
Comparison of the predicative accuracy and the goodness of fitting between the traditional TNM models and adjusted models with NLR in gastric cancer patients.

| Model | C-index | AIC |
|---|---|---|
| TNM model1:<br>pTNM stage | 0.681 | 5536 |
| Adjusted model1:<br>pTNM stage + NLR | 0.698 | 5513 |
| TNM model2:<br>pTNM stage + gender + CD3 + Lauren + PDL1 | 0.722 | 5492 |
| Adjusted model2:<br>pTNM stage + gender + CD3 + Lauren + PDL1 + NLR | 0.725 | 5478 |
| AIC: Akaike information criterion | | |

Analysis of the time-dependent ROC curves at five years for OS showed that the AUCs of the NLR-adjusted model were larger than those of the TNM model in both model 1 and model 2 (Fig. 3B and 3C). The C-index showed a good predictive accuracy for NLR-adjusted model in model 1 and model 2 (Table 3). In terms of the goodness of fitting, the NLR-adjusted model (AIC: 5513 in model 1; 5478 in model 2) was better than the TNM model (AIC: 5536 in model 1; 5492 in model 2) (Table 3). These results indicated that the NLR was able to improve the predictive accuracy of TNM model in either univariable or multivariable model, and the NLR could avoid penalty for complex models due to overfitting.

# Mediation analysis

According to the results of univariable and multivariable COX regression analyses, the mediator effect of TCI might exist among the relationship among NLR and OS, as following facts: (1) TCI was significantly correlated with the NLR ($P < 0.05$) and survival ($P < 0.05$), (Table 1 and Table 2); (2) NLR was significantly associated with OS ($P < 0.001$) in univariable analysis (Table 2), (3) the correlation intensity between NLR and OS weakened after controlling TCI in multivariable analysis (Table 2). Mediation analysis indicated that CD3 + TCI had significant mediation effects on the association between NLR and overall survival ($P < 0.001$), which explains about 9.21% of the association (Fig. 4). CD3 + TCI is a mediator of the association between NLR and overall survival.

# Discussion

We revealed the prognostic value of NLR, an indicator of systemic inflammation, in gastric cancer. The NLR-adjusted pTNM staging had a better predictive value for patient prognosis when compared with the traditional pTNM staging system. In addition, a higher NLR was associated with advanced-stage gastric

cancer. Infiltration of CD3 + and CD8 + T cells was significantly different in patients with different levels of NLR. These findings indicate that systemic inflammation involves in the development and progression of gastric cancer. The peripheral blood NLR could be used to reflect the cancer-immune microenvironment and to predict the patient with a worse prognosis who might need aggressive postoperative adjuvant therapy.

In recent years, our understanding of the cancer-immune microenvironment has greatly improved. Lymphoid regulatory cells include regulatory T, B, and NK cells in the tumor site, have been reported to support cancer growth, migration, and metastasis [14]. Cancer-associated myeloid cells are involved in cancer cell biology, including proliferation, invasion, distant metastasis, and the development of resistance to therapy [15]. STAT3 played a role in several crosstalk levels between tumor cells and the immune microenvironment, and mediated tumor-induced immunosuppression [16]. Helicobacter pylori (HP) infection-induced chronic inflammation is a significant factor for gastric cancer [17]. The development of gastric cancer is attributed to genetic alterations caused by chronic inflammation, recruitment of immune cells, an imbalance between epithelial cell proliferation and apoptosis, and gastric colonization by enteric bacteria. Increased expression of pro-inflammatory cytokines and chemokines such as IL-17A, IL-22, and IL-1 family members IL-1β are involved in gastric cancer progression [18]. Thus, systemic inflammation might play an essential role in the gastric cancer microenvironment. Besides these pieces of molecular biology evidence, our results provided more information about systemic inflammation and tumor microenvironment from the perspective of clinical prognosis through mediation analysis.

Lymphopenia is an impaired cell-mediated inflammatory response, whereas neutrophilia is an immune response that triggers tumorigenesis [19]. NLR can be used to represent a balance between pro-tumor and antitumor immunity. The NLR also can reflect the cancer burden and tumor biological behavior. Kei Nakamura, et al showed that NLR was associated with undifferentiated histology, advanced clinical T stage and N stage [20]. Kazuhiro Migita, et al concluded NLR might be associated with the extent of tumor spread at the time of recurrence [21]. Consistent with previous studies, we found that a high level of NLR was associated with the late tumor stage. The TNM stage system is always be used to predict the prognosis of cancer and guide postoperative treatment. Nevertheless, prognosis differs greatly among patients with the same tumor stage, which indicates that other prognostic factors should be included to optimize the system. Previous studies have reported the prognostic value of NLR in gastric cancer with the cutoff value set as 3 mg/dL [11, 22, 23]. Similarly, in our study, patients were also divided into two groups based on the level of NLR. The NLR-adjusted pTNM staging system allowed more accuracy to predict the prognosis of gastric cancer. NLR is an independent prognostic predictor of gastric cancer. Taken together, we conclude that NLR can be proposed and used as a predictor to stratify patients with different prognosis.

Lymphocytes are associated with a favorable prognosis in multiple tumors. The NLR is associated with the density of CD4 + T cells in gastric cancer [11]. It also negatively correlated with CD8 + T cells infiltrating in biliary tract cancer [24]. High NLR is significantly associated with high neutrophil infiltration

and low CD3 + T cells in glioblastoma [25]. Here, we showed that the infiltration of CD3 + and CD8 + T cells was both significantly different in patients with different levels of NLR. The infiltration level of CD3 + T cells was the mediating factor between NLR and survival prognosis. Thus, the NLR might serve as a valuable indicator for evaluating the immunoreactivity in the gastric cancer microenvironment.

The NLR was thought to be correlated with the tolerability and response to anticancer therapy in multiple cancers [26–28]. NLR might be valuable for screening patients who will benefit from palliative chemotherapy. In our study, 88.2% of patients were with locally advanced/advanced gastric cancer. Many of them were treated with postoperative chemotherapy after D2 radical gastrectomy. Surprisingly, the effect of NLR on the patient prognosis prediction was affected by MMR status. MMR status is a mature biomarker for predicting the efficacy of immune checkpoint inhibitors. This might indicate that the NLR level is associated with the efficacy of immune therapy and that markers of systemic inflammation could be used to predict immune responses in cancer therapy.

There are some limitations in this study. Firstly, this is a retrospective study in which patient selection bias might exist. Secondly, the cutoff value of NLR is needed to be verified in a multicentric, independent cohort. Thirdly, we did not evaluate the postoperative dynamic changes in the NLR. Lastly, the assessment of some reported systemic inflammation indicators, such as CRP, was not included in this study. Larger prospective studies are needed to evaluate these issues. However, despite the limitations mentioned above, we evaluated the mediation effects of tumor microenvironment on the association between systemic inflammation and overall survival for the first time. In addition, we demonstrated that the NLR was able to improve the predictive accuracy of the TNM model and describe the relationship between NLR and immune checkpoints.

# Conclusions

In summary, we revealed that the level of NLR is an independent prognostic predictor of gastric cancer. Adding an index representing the level of NLR can improve the prognostic prediction value of the TNM staging system in gastric cancer patients. The effect of NLR on patient prognosis is partly through influencing CD3 + T cells infiltration.

# Abbreviations

GC: Gastric cancer; NLR: Neutrophil-to-lymphocyte ratio; TIL: Tumor-infiltrating lymphocytes; TLS: Tertiary lymphoid structures; PDL1: Programmed cell death-ligand 1; HP: Helicobacter pylori; MMR: Mismatch repair; AIC: Akaike Information Criterion; ROC: Receiver operating characteristic curve; DCA: Decision curve analysis; EGJ: Esophagogastric junction; HR: Hazard ratio; CI: Confidence interval; ACME: Average causal mediation effects; ADE: Average direct effects.

# Declarations

## Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and had been authorized by the Ethics Committee of Peking University Cancer Hospital.Written informed consents from voluntary subjects were obtained.

## Consent for publication

Not applicable.

## Availability of data and materials

The data that support the findings of this study are available from Peking University Cancer Hospital but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Peking University Cancer Hospital.

## Competing interests

The authors declare that they have no competing interests.


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## Authors’ Contributions

XXF and YW conceived and designed the study. The data collection and analyses were conducted by HQF, ZQZ, FB, HL and LL. HQF and ZQZ wrote the manuscript. All authors have read and approved the manuscript.


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None.


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## Supplemental Figure

Supplemental Figure 1 is not available with this version

## Figures

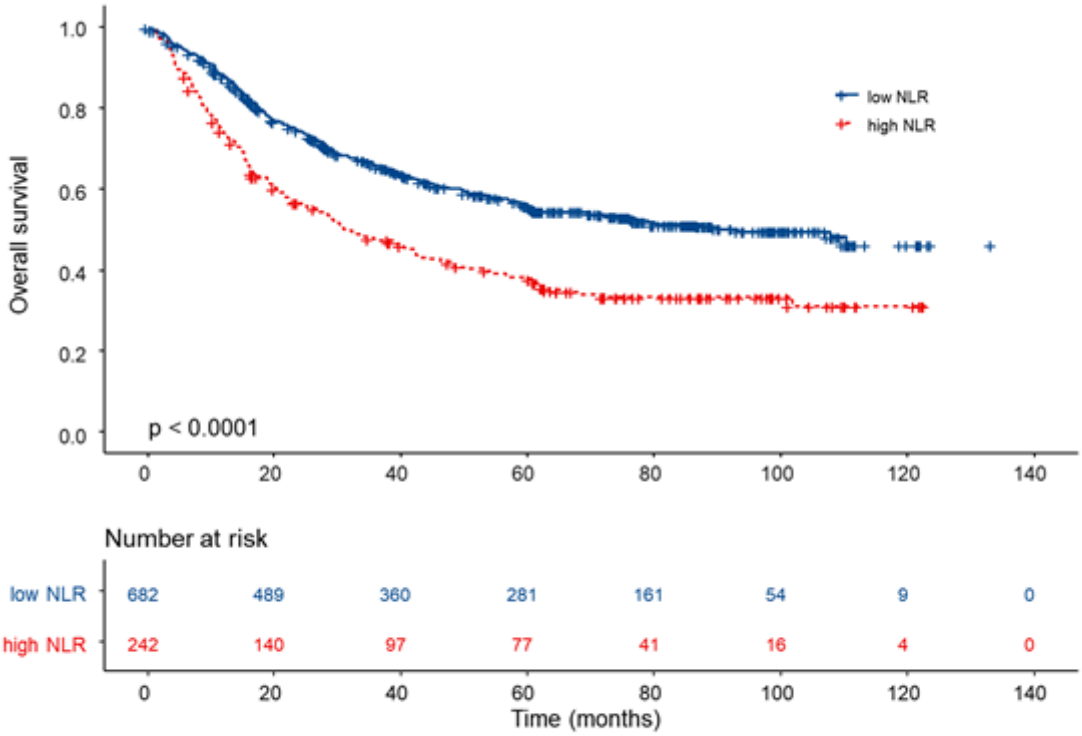
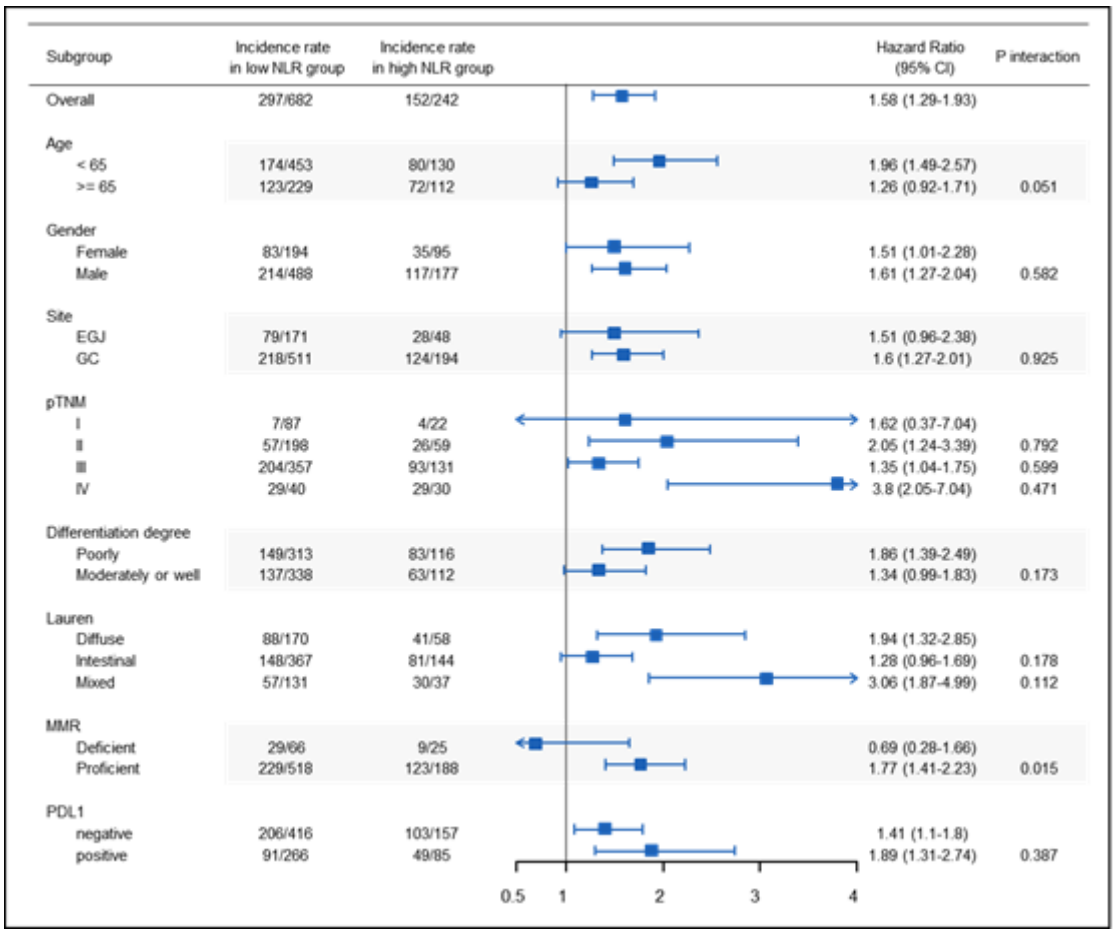


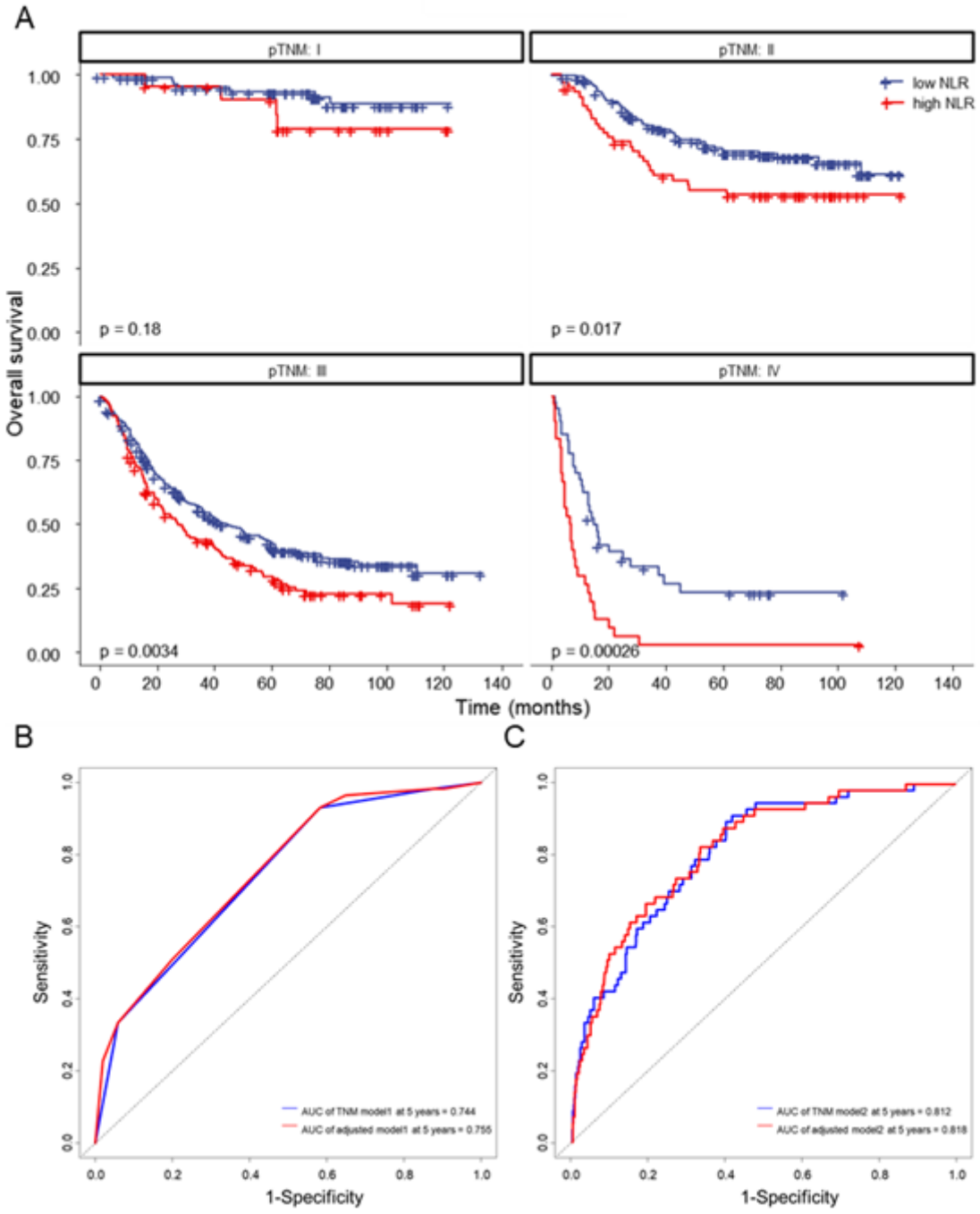

**Figure 1**

Kaplan-Meire curves of GC patients with different NLR levels.




## Figure 2

Forest plot of overall survival stratified by subgroups. Subgroup analyses and interaction analyses were conducted using patients' baseline characteristics.




## Figure 3

NLR is an independent prognostic factor and able to improve the predictive value of the TNM staging system. A. Kaplan-Meier curves stratified by pTNM staging, NLR was a prognostic predictor independent from TNM staging system; B & C. Receiver operating characteristic detect the predictive value of NLR in gastric cancer prognosis in two models with or without NLR. The model 1 was TNM multivariable Cox model based on the pTNM stage, age, CD3+ T cell infiltration, Lauren classification and PDL1 expression, which were identified as the independent prognostic factors of gastric cancer. The model 2 was TNM univariable Cox model.

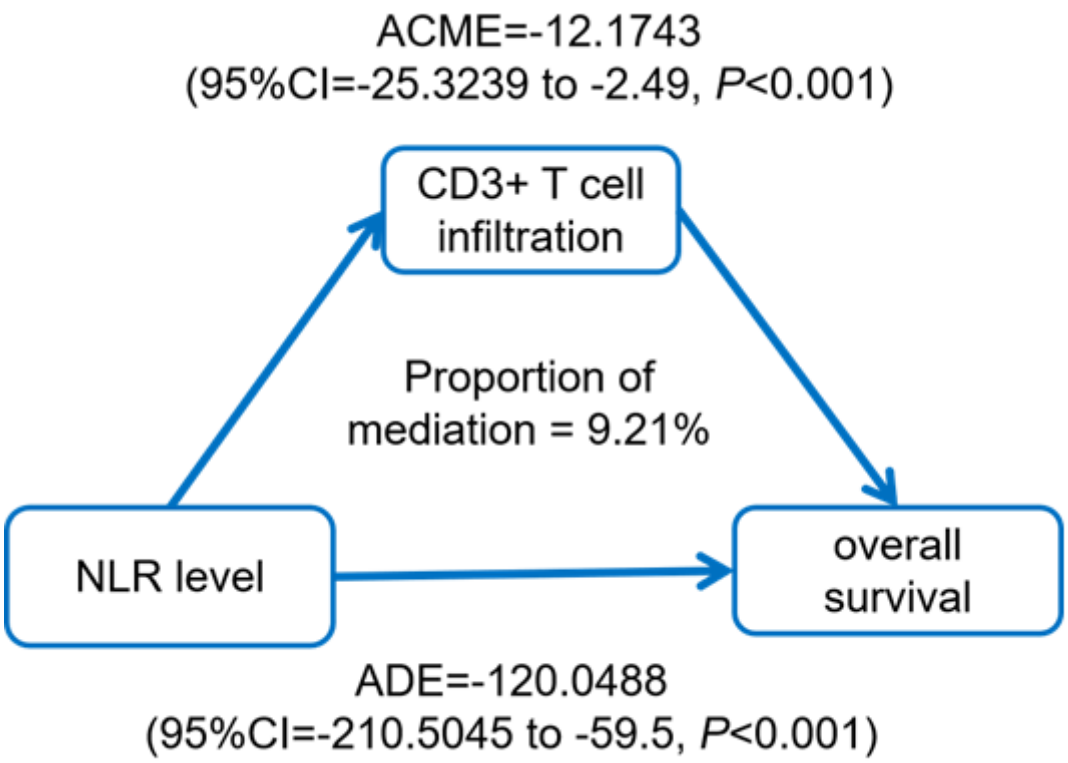



**Figure 4**

A Causal Mediation Analysis of the CD3+ T cell infiltration Between NLR and OS. ACME: average causal mediation effects; ADE: average direct effects.