

Research Paper

Elevated preoperative neutrophil-to-lymphocyte ratio can predict poor survival in early stage gastric cancer patients receiving radical gastrectomy: The Fujian prospective investigation of cancer (FIESTA) study

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Abstract

Aims: This cohort study was conducted to evaluate the prognostic impact of blood-routine parameters before radical gastrectomy on gastric cancer mortality.

Methods: Total 3012 patients with gastric cancer were consecutively enrolled from a mono-center between 2000 and 2010, and the latest follow-up was completed in 2015.

Results: The median follow-up time was 44.05 months. Finally, 1331 out of 3012 gastric cancer patients died from gastric cancer. Per standard deviation increment in neutrophil (hazard ratio or HR=1.08, P<0.001), white blood cell count (HR=1.07, P=0.001), neutrophil-to-lymphocyte ratio or NLR (HR=1.08, P<0.001) and platelet-to-lymphocyte ratio (HR=1.08, P<0.001) was significantly associated with an increased risk of gastric cancer mortality, while that in lymphocyte (HR=0.69, P<0.001), hemoglobin (HR=0.82, P<0.001) and lymphocyte-to-monocyte ratio (HR=0.68, P<0.001) was associated with a reduced risk. Survival tree analysis indicated that in patients with TNM stage I/II, the contrasts of NLR>2.61 with ≤2.61 and NLR>1.87 with ≤1.87 were respectively associated with a 5.21-fold (P=0.004) and 2.36-fold (P=0.001) increased risk of gastric cancer mortality. The effect-size magnitude of NLR was further potentiated in patients with invasion depth T1/T2 (HR=1.73, P=0.001), regional lymph node metastasis N0 (HR=1.60, P<0.001), TNM stage I/II (HR=1.36, P=0.009) and tumor size ≤ 4.5 cm (HR=1.17, P<0.001).

Conclusions: Our findings consolidated the prognostic impact of preoperative NLR on gastric mortality, and demonstrated that elevated preoperative NLR was a robust indicator of poor survival in patients at early stage.

Key words: Gastric cancer; Blood-routine parameter; Prognosis; Survival.

Introduction

Gastric cancer tops the incidence of malignancies and is the leading cause of cancer mortality worldwide [1]. In China, it is projected that 679 100

new cases and 498 000 deaths from gastric cancer occurred during the year 2015 [2]. A long-existing dilemma facing clinical doctors is that the majority of

patients with gastric cancer are diagnosed at high-grade invasive stages, mainly due to the lack of obvious symptoms, clinical indications and effective surveillance programs. Moreover, the overall 5-year survival rate of gastric cancer revolves around 20% in China [3]. An effective target for early identification and intervention of gastric cancer will be high on the agenda. One of the most urgent tasks before us is to seek some prognostic biomarkers that can assist in improving the clinical management of gastric cancer patients.

Recent two decades have witnessed some major achievements in identifying gastric cancer-specific biomarkers in scientific research and medical applications; however, no general agreement exists yet as to the clinical benefits of any single biomarkers for the diagnosis, prognosis and therapy of gastric cancer [4-8]. One of possible reasons is that most conclusions in current literature are based on underpowered studies that are prone to false-positive errors [9]. Besides, diversity in population characteristics such as dietary habits and lifestyles may also add up. For instance, high-sodium diets and cigarette smoking are established as major contributing factors for the development of gastric cancer [10-12]. For practical reasons, a growing number of studies have interrogated the association of blood-routine parameters with the risk and prognosis of gastric cancer [13-16]. It is worth mentioning that the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are widely evaluated and their prognostic capability for high-risk gastric cancer has been verified in many cohort studies [17-19]. In spite of exhaustive research regarding this subject, it still remains elusive whether the prognostic impact of these significant parameters on gastric cancer varies across distinct clinicopathologic settings. Further optimizing the cutoff value(s) of significant parameter(s) poses a formidable challenge. A traditional method to determine an optimal cutoff value relies on the receiver-operator characteristic analysis, while its incapability to consider survival time hampers the discrimination utility, leading to an inaccurate classification. To overcome this limitation and yield more information, we aimed to evaluate the prognostic impact of blood-routine parameters before radical gastrectomy on gastric cancer mortality among 3012 patients over a 15-year follow-up period by eliciting a subset of the data from the ongoing Fujian prospective investigation of cancer (FIESTA) study [20-24]. In addition, we adopted survival tree analysis to determine the optimal cutoff value of the most significant parameter and further test its prognostic specificity across patients within different

clinicopathologic profiles.

Methods

Study patients

All study patients were obtained from the ongoing FIESTA study initiated in January 2000. The FIESTA study is an ongoing prospective cohort study of common digestive system tumors, including esophageal cancer, gastric cancer and colorectal cancer [20-24], and the chief objective of the FIESTA study is attempting to look for preoperative risk factors for cancer-specific mortality, which will assist in defining rational targets to delay tumor progression and prolong the survival of cancer patients after surgery. For this study, we consecutively collected preoperative patients with operable gastric cancer until December 2010 from the Department of Thoracic Surgery, Fujian Provincial Cancer Hospital. The latest follow-up was finished in December 2015. All study patients gave written informed consent to denoting blood and tissue samples for clinical assays and to being contacted during follow-up. The ethical committees of Fujian Provincial Cancer Hospital approved this study. All study protocols were implemented in accordance with the relevant guidelines and regulations.

Tissue collection and diagnosis

Each patient agreed to provide a pair of cancer and normal tissue samples, which were immediately fixed by 10% neutral formalin and embedded in paraffin. The clinicopathologic characteristics were determined at the Department of Pathology, Fujian Provincial Cancer Hospital.

Eligibility criteria

Eligible gastric cancer patients must simultaneously meet the following criteria: (i) they were of Han Chinese nationality; (ii) they had no consanguinity at enrollment; (iii) they were firstly hospitalized for radical gastrectomy; (iv) they received no preoperative/postoperative chemotherapy or radiotherapy; (v) they must be followed up for 1 month and more; (vi) they must have gastric cancer confirmed by preoperative biopsy or postoperative pathologic tests.

Follow-up assessment

After radical gastrectomy, all patients were requested to be rechecked every six months at the Out-Patient Department, Fujian Provincial Cancer Hospital, and in case of no-show at the reserved time they were contacted through calling or sending post mails. We followed each patient up from the initial enrollment date to death time or the last checking

point during 2015, whichever came first, and survival period was transformed into months for survival analysis.

Blood-routine parameters

In the morning of surgery day, fasting 4 mL venous blood samples were collected into an EDTA-K2 anticoagulative tube for measuring blood-routine parameters, including neutrophil, lymphocyte, monocyte, eosinophil, basophil, white blood cell count, red blood cell count, hemoglobin, red cell distribution width and platelet count by the SYSMEX XE-2100 Automatic Blood Cell Analyzer (Sysmex, Kobe, Japan) at the Clinical Laboratory, Fujian Provincial Cancer Hospital. The period from blood drawing to clinical analysis was less than 4 hours according to a standard procedure. In addition, based on single blood-routine parameters, we calculated NLR, PLR and lymphocyte-to-monocyte ratio (LMR) accordingly.

Demographic and clinicopathologic characteristics

At the time of enrollment, we invited each patient to complete a self-designed questionnaire to record demographic information, including date of birth, age of onset for gastric cancer, gender, body weight, body height, smoking, drinking and family cancer history. Age was defined as the age at the time of operation for gastric cancer. Body mass index was calculated as weight (in kilograms) divided by the square of height (in meters). Smoking status was classified as never smoking and ever smoking (including formerly and currently smoking). Drinking status was classified as never drinking and ever drinking (including formerly and currently drinking).

Clinicopathologic data were gathered immediately after radical gastrectomy from medical charts and pathological reports, including tumor-node-metastasis (TNM) stage (I, II, III and IV according to the 7th Edition of the UICC/AJCC TNM Staging System [25]), tumor size (in centimeters), depth of invasion (T1, T2, T3 and T4), regional lymph node metastasis (N0, N1, N2 and N3), distant metastasis (M0 and M1), the Lauren's classification (intestinal type and diffuse type) and embolus (positivity and negativity).

Statistical analysis

Continuous variables expressed as median (inter-quartile range) were compared between non-survivors and survivors by the Mann-Whitney U test, and categorical variables expressed as percentage were compared by the Chi-squared test. Both continuous and categorical variables were analyzed as their original forms in the following analyses. The

Kaplan-Meier method was used to estimate survival time and the Log-rank test was used for survival comparisons. The prediction of blood-routine parameters for gastric cancer mortality was expressed as hazard ratio (HR) and its 95% confidence interval (95% CI) by using the multivariate Weibull proportional hazards regression model. The STREE program (<http://c2s2.yale.edu/software/stree/>) was adopted to determine the optimal cutoff values by splitting patients with significantly differed median survival time (MST). Unless otherwise stated, all statistical tests are two-sided and are significant at a level of 5%, and the STATA software (StataCorp, TX, USA, version 13.0) was used for statistical analysis.

Results

Baseline characteristics

In total, this study involved 3413 gastric cancer patients. Due to the loss to follow-up (n=318), failure to survive over 1 month (n=48) and death of causes other than gastric cancer (n=35), we finally had 3012 gastric cancer patients with complete data for survival analysis. The follow-up time ranged from 1.1 to 183.3 months and the median was 44.05 months. As of late 2015, 1331 out of 3012 gastric cancer patients were recorded to die from gastric cancer.

The comparisons of demographic and clinicopathologic characteristics, as well as blood-routine parameters are shown in Table 1. Non-survivors were older than survivors at enrollment (median: 60 years vs. 58 years, $P<0.001$). In addition, baseline body mass index was higher in non-survivors than in survivors ($P=0.007$). There were no differences in male percentage ($P=0.144$) between the two groups, as well as in the percentages of smoking ($P=0.212$), drinking ($P=0.643$) and family cancer history ($P=0.214$). The median levels of neutrophil, white blood cell count, NLR and PLR were significantly higher in non-survivors than survivors, while that of lymphocyte, hemoglobin and LMR were significantly lower ($P<0.05$). No significance difference was noted for the other blood-routine parameters under study. Further for clinicopathologic characteristics, the distributions of invasion depth, regional lymph node metastasis, distant metastasis, TNM stage, Lauren's classification and embolus differed significantly between the two groups ($P<0.001$ for all).

Prediction of blood-routine parameters

The risk effect-size estimates of blood-routine parameters for gastric cancer mortality per standard deviation (SD) increment are summarized in Table 2. Considering the facts that the mortality rate of gastric cancer increases smoothly over time and the

$\ln(-\ln(S(t)))$ is a linear function of $\ln(t)$ (here, t is survival time, and $S(t)$ is survival function), it is more appropriate to adopt the Weibull proportional hazards model for multivariate-adjusted survival analysis. As compared with the crude results, we controlled for age, gender, body mass index, smoking, drinking and family-cancer history under the multivariate Weibull proportional hazards regression model. Per SD increment in neutrophil (adjusted HR, 95% CI, P: 1.08, 1.05-1.11, <0.001), white blood cell

count (1.07, 1.03-1.11, 0.001), NLR (1.08, 1.06-1.12, <0.001) and PLR (1.08, 1.05-1.11, <0.001) was associated with a significantly increased risk for gastric cancer mortality, respectively, even after adjusting for confounding factors, while per SD increment in lymphocyte (0.69, 0.62-0.77, <0.001), hemoglobin (0.82, 0.78-0.87, <0.001) and LMR (0.68, 0.59-0.80, <0.001) was significantly associated with a reduced risk. A marginally increased risk was noted for platelet count (1.06, 1.00-1.13, 0.036).

Table 1. The baseline characteristics of cohort patients by primary clinical outcome

Characteristics	Non-survivors (n=1331)	Survivors (n=1681)	P
Age (years)	60 (52, 68)	58 (51, 66)	<0.001
Males	73.03%	75.37%	0.144
Body mass index (kg/m ²)	22.72 (20.63, 25.21)	22.5 (20.70, 24.44)	0.007
Smoking	18.96%	20.87%	0.212
Drinking	5.82%	6.24%	0.643
Family cancer history	8.53%	9.91%	0.214
Neutrophil (10 ⁹ /L)	3.9 (3.0, 5.0)	3.6 (2.7, 4.6)	<0.001
Lymphocyte (10 ⁹ /L)	1.7 (1.3, 2.1)	1.9 (1.5, 2.3)	<0.001
Monocyte (10 ⁹ /L)	0.5 (0.4, 0.6)	0.5 (0.4, 0.6)	0.225
Eosinophil (10 ⁹ /L)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.128
Basophil (10 ⁹ /L)	0 (0, 0)	0 (0, 0)	0.043
White blood cell count (10 ⁹ /L)	6.4 (5.3, 7.7)	6.3 (5.1, 7.6)	0.005
Red blood cell count (10 ¹² /L)	4.1 (3.6, 4.48)	4.3 (3.8, 4.62)	0.914
Hemoglobin (g/L)	120 (98, 134)	130 (111, 142)	<0.001
Platelet count (10 ⁹ /L)	253 (204, 317)	251 (202, 307)	0.059
Red cell distribution width (%)	13.4 (12.5, 15.3)	13.1 (12.4, 14.2)	0.169
Neutrophil-to-lymphocyte ratio	2.27 (1.70, 3.06)	1.86 (1.38, 2.61)	<0.001
Platelet-to-lymphocyte ratio	151.3 (110.9, 204.9)	133.7 (101.1, 180.0)	<0.001
Lymphocyte-to-monocyte ratio	3.3 (2.5, 4.3)	3.8 (2.9, 5.0)	<0.001
Invasion depth			<0.001
T1	1.00%	15.58%	
T2	3.69%	13.38%	
T3	54.46%	55.85%	
T4	40.85%	15.19%	
Regional lymph node metastasis			<0.001
N0	9.69%	40.89%	
N1	30.31%	32.43%	
N2	44.31%	23.45%	
N3	15.69%	3.23%	
Distant metastasis			<0.001
Negative	74.75%	98.58%	
Positive	25.25%	1.42%	
Tumor-node-metastasis stage			<0.001
I	1.23%	21.20%	
II	7.41%	21.20%	
III	59.34%	54.75%	
IV	32.02%	2.84%	
The Lauren's classification			<0.001
Intestinal type	30.60%	45.76%	
Diffuse type	69.40%	54.24%	
Embolus			<0.001
Negative	49.88%	70.22%	
Positive	50.12%	29.78%	
Tumor size (cm)	6.0 (4.5, 8.0)	4.0 (3.0, 6.0)	<0.001

Data are expressed as median (interquartile range) or percentage. P was calculated by the Mann-Whitney U test or the Chi-squared test where appropriate.

Table 2. The risk effect-size estimates of blood routine parameters for gastric cancer mortality

Blood routine indexes	Increment (s. d.)	HR, 95% CI, P	HR, 95% CI, P*
Neutrophil (10 ⁹ /L)	2.40	1.08, 1.05-1.11, <0.001	1.08, 1.05-1.11, <0.001
Lymphocyte (10 ⁹ /L)	1.08	0.67, 0.60-0.74, <0.001	0.69, 0.62-0.77, <0.001
Monocyte (10 ⁹ /L)	0.42	1.02, 0.97-1.07, 0.439	1.01, 0.97-1.07, 0.559
Eosinophil (10 ⁹ /L)	0.24	0.94, 0.87-1.01, 0.083	0.94, 0.87-1.01, 0.110
Basophil (10 ⁹ /L)	0.22	1.03, 0.98-1.07, 0.230	1.03, 0.99-1.07, 0.198
White blood cell count (10 ⁹ /L)	2.87	1.07, 1.03-1.11, 0.001	1.07, 1.03-1.11, 0.001
Red blood cell count (10 ¹² /L)	16.58	0.98, 0.91-1.07, 0.714	0.99, 0.91-1.07, 0.820
Hemoglobin (g/L)	28.73	0.82, 0.78-0.86, <0.001	0.82, 0.78-0.87, <0.001
Platelet count (10 ⁹ /L)	96.56	1.06, 1.00-1.12, 0.041	1.06, 1.00-1.13, 0.036
Red cell distribution width (%)	16.58	1.04, 0.99-1.09, 0.111	1.04, 0.99-1.09, 0.109
Neutrophil-to-lymphocyte ratio	2.63	1.09, 1.06-1.12, <0.001	1.08, 1.06-1.12, <0.001
Platelet-to-lymphocyte ratio	106.65	1.08, 1.05-1.11, <0.001	1.08, 1.05-1.11, <0.001
Lymphocyte-to-monocyte ratio	3.89	0.65, 0.56-0.75, <0.001	0.68, 0.59-0.80, <0.001

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval. The effect-size estimates were calculated under the Weibull proportional hazards regression models. *P was adjusted for age, gender, body mass index, smoking, drinking and family cancer history.

Table 3. Risk classification of TNM stage based on survival tree analysis and the effect-size estimates for gastric cancer mortality

TNM stage	Comparison	HR, 95% CI, P	HR, 95% CI, P*
I	NLR: >2.61 vs. ≤2.61	8.48, 3.02-23.83, <0.001	5.21, 1.71-15.90, 0.004
II	NLR: >1.87 vs. ≤1.87	2.76, 1.74-4.38, <0.001	2.36, 1.45-3.85, 0.001
III	LNM: >9 vs. ≤9	2.43, 2.08-2.83, <0.001	2.51, 2.12-2.98, <0.001
IV	Tumor size (cm): >4.5 vs. ≤4.5	1.81, 1.39-2.36, <0.001	1.75, 1.33-2.31, <0.001

Abbreviations: TNM, tumor node metastasis; NLR, neutrophil-to-lymphocyte ratio; LNM, the number of regional lymph node metastasis; HR, hazard ratio; 95% CI, 95% confidence interval. The effect-size estimates were calculated under the Weibull proportional hazards regression models. *P was adjusted for age, gender, body mass index, smoking, drinking and family cancer history.

Survival tree analysis

Displayed in Figure 1 is the survival tree structure over blood-routine parameters, demographic and clinicopathologic characteristics. The top splitting factor was TNM stage, and the MST in patients with TNM stage III-IV was half of that in patients with TNM stage I-II (33.0 months vs. 68.3 months, $P < 0.001$). The discrimination ability of NLR was secondary to that of TNM in both patients with stage I and II. In contrast to patients with TNM stage III and IV, regional lymph node metastasis and tumor size served as the second splitting factors, respectively. To enhance statistical power, only nodes involving at least 100 patients were numbered and a total of 10 nodes were selected, and their discrimination ability for gastric cancer mortality was further embodied in the Kaplan-Meier curves (Figure 1).

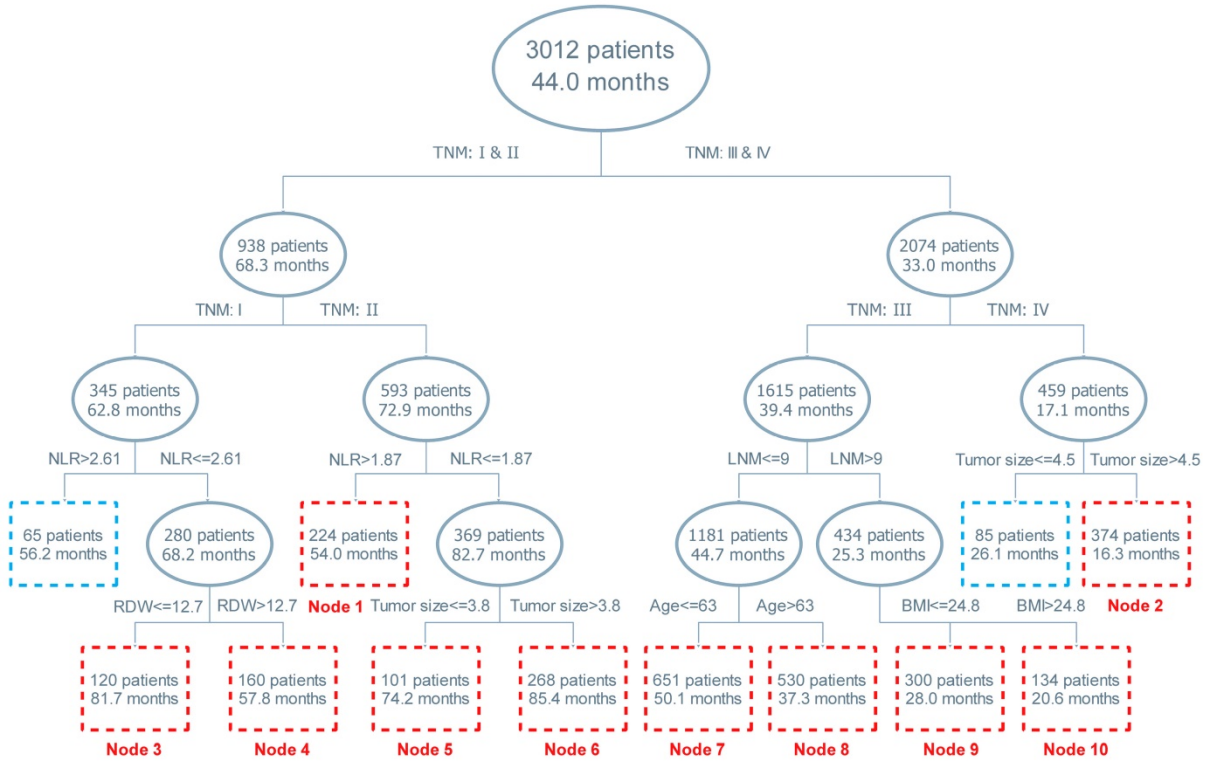
Given the importance of TNM stage in survival tree analysis, we calculated the risk effect-size estimates of the secondary splitting factor within each stage (Table 3). In patients with TNM stage I and II, the contrast of NLR >2.61 with ≤2.61 and NLR >1.87 with ≤1.87 was respectively associated with a 5.21-fold (adjusted HR, 95% CI, P: 5.21, 1.71-15.90, 0.004) and 2.36-fold (2.36, 1.45-3.85, 0.001) increased risk of gastric cancer mortality after adjusting for

confounders. In addition for patients with TNM stage III and IV, the contrast of regional lymph node metastasis number >9 with ≤9 and tumor size >4.5 cm with ≤4.5 cm was respectively with a 2.51-fold (2.51, 2.12-2.98, <0.001) and 1.75-fold (1.75, 1.33-2.31, <0.001) increased risk after adjustment.

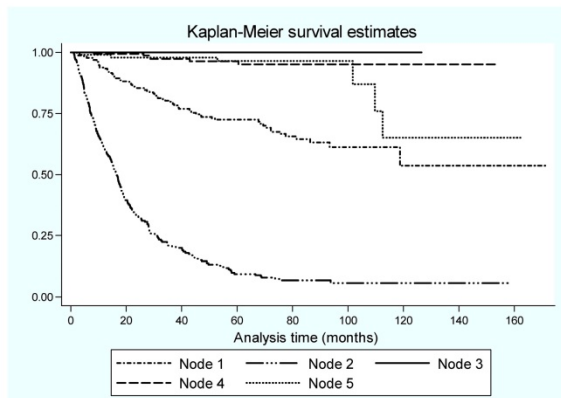
Subgroup analysis

In view of the significant discrimination ability of NLR in survival tree analysis, we further examined its risk effect-size estimates per SD increment for gastric cancer mortality according to clinicopathologic characteristics, as shown in Table 4. The magnitude of effect-size estimates of NLR was potentiated in patients with invasion depth T1-T2 (adjusted HR, 95% CI, P: 1.73, 1.25-2.40, 0.001), regional lymph node metastasis N0 (1.60, 1.34-1.90, <0.001), TNM stage I-II (1.36, 1.08-1.70, 0.009), positive embolus (1.19, 1.11-1.28, <0.001) and tumor size ≤4.5 cm (1.17, 1.07-1.28, <0.001) relative to patients with invasion depth T3-T4, regional lymph node metastasis N1-N3, TNM stage III-IV, negative embolus and tumor size >4.5 cm, respectively. In addition for the Lauren's classification, the multivariate HRs were 1.18 (95% CI, P: 1.12-1.25, <0.001) and 1.06 (1.01-1.12, 0.026) for the diffuse and intestinal types, respectively. No difference was noticed for patients with positive and negative metastases.

(A)



(B)



(C)

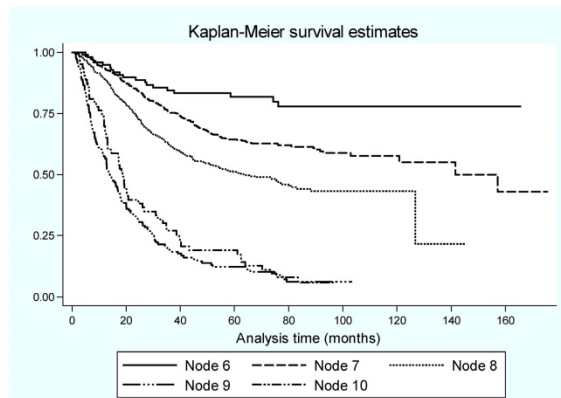


Figure 1. Survival tree structure of blood-routine parameters along with demographic and clinicopathological characteristics. Abbreviations: NLR, Neutrophil-to-lymphocyte ratio; LNM, lymph node metastasis; RDW, red cell distribution width; BMI, body mass index. Panel B and C are the Kaplan-Meier curves of different nodes generated by survival tree analysis (panel A). In panel A, the upper number in the box refers to the number of patients, and the lower number refers to the median survival time.

Discussion

Via a 15-year prospective analysis of preoperative blood-routine parameters for the mortality of 3012 patients, our findings consolidated the prognostic value of preoperative NLR and demonstrated that elevated NLR was a robust indicator of poor survival in patients with early stage gastric cancer receiving radical gastrectomy. In addition, we adopted survival tree analysis and further optimized the discrimination accuracy of NLR by recommending cutoff values in patients with TNM

stage I and II. To the best of our knowledge, this is to date the largest cohort study that has evaluated the prognostic significance of blood-routine parameters before the operation to predict gastric cancer mortality.

A recent meta-analysis by Sun et al who pooled the results of 19 studies and 5431 patients indicated that elevated pretreatment NLR was a significant predictor of poor outcomes for gastric cancer patients, particularly at late stage [17]. Consistent with the findings of this meta-analysis [17], we in an analysis of 3012 gastric cancer patients for radical gastrectomy

reported that per SD increment in preoperative NLR was significantly associated with an 8% increased risk of gastric cancer mortality, independent of age, gender, obesity, smoking and drinking habits. It is also worth noting that, inconsistent with the meta-analytical findings by Sun et al [17], our subgroup analysis and survival tree analysis demonstrated that the discrimination ability of NLR for postoperative mortality was strongly potentiated in gastric cancer patients at TNM stage I and II rather than at stage III and IV, as well as in patients with low depth of invasion, no detectable lymph node metastasis and small tumor size, respectively. The major reason for this inconsistency may be due to the divergent lengths of the follow-up period. In the current cohort study, we consecutively enrolled gastric cancer patients for surgical treatment from 2000 to 2010 at the Fujian Provincial Cancer Hospital, and the latest follow-up was completed in late 2015. Such a long follow-up period allowed us to accurately assess the clinical outcomes of most gastric cancer patients in this study. Over 15-year follow-up, we found that 415 (90.41%) out of 459 patients with TNM stage IV died of gastric cancer. By contrast to the meta-analysis by Sun et al [17], the median follow-up time ranged from 9.3 months [26] to 96 months [27] and it is possible that in studies with a shorter follow-up period, the clinical outcomes of gastric cancer patients at advanced stage haven't happened yet by the end of follow-up, causing an overestimation of the true effect of NLR in prognosis. In addition, differences in age, gender composition, treatment modality, tumor size and tumor differentiation might also introduce a selection bias in the Sun et al meta-analysis [17]. We agree that confirmation of our prospective findings in another independent large cohort is critical. Nevertheless, considering the potential implications of preoperative NLR in predicting mortality, we maintain that the evaluation of blood-routine parameters for gastric cancer patients receiving radical gastrectomy, especially at an early stage, will facilitate risk stratification and the identification of high-risk patients for targeted preventative and therapeutic interventions.

Extending previous findings on this topic, we additionally recommended the optimal cutoff values for preoperative NLR in patients with early stage gastric cancer by employing survival tree analysis, and the prognostic utility of these cutoff values was further validated by the Weibull proportional hazards regression model (Table 3). Moreover, what has attracted our attention most in survival tree analysis is that the prognostic utility of NLR in gastric cancer patients with TNM stage I-II is as fairly important as

that of regional lymph node metastasis and tumor size respectively in patients with TNM stage III and IV, highlighting the robustness of NLR as a early prognostic indicator for gastric cancer mortality. Although the exact mechanism behind this prognostic significance of NLR remains to be elucidated, it has been proposed that the dominant pro-tumor activities of neutrophils and reduced anti-tumor immune response by lymphocytes, as denoted by NLR, may have an impact on poor tumor response and unfavorable prognosis [28-30]. As supported by several lines of evidence, neutrophilia is the most sensitive responder to the inflammatory activity of the tumor, and elevated neutrophils in circulation can promote tumor growth and metastasis or suppress lymphocyte activity, thereby counteracting the antitumor immune response [16, 31-33]. In contrast, lymphocytopenia can reflect the depression of innate cellular immunity indicated by a marked decrease in T4 helper lymphocytes and an increase in T8 suppressor lymphocytes [34]. As such, it is reasonable to speculate that elevated NLR might, if involved, induce the inflammation/immune response and anti-tumor activities to be associated with poor prognosis of gastric cancer patients.

Table 4. The effect-size estimates of neutrophil-to-lymphocyte ratio for gastric cancer mortality according to clinicopathological characteristics

Subgroups	HR, 95% CI, P	HR, 95% CI, P*
Invasion depth		
T1-T2	1.66, 1.24-2.23, 0.001	1.73, 1.25-2.40, 0.001
T3-T4	1.07, 1.04-1.10, <0.001	1.07, 1.03-1.10, <0.001
Regional lymph node metastasis		
N0	1.71, 1.47-1.99, <0.001	1.60, 1.34-1.90, <0.001
N1-N3	1.06, 1.03-1.09, <0.001	1.06, 1.03-1.10, 0.001
Distant metastasis		
Positive	1.08, 0.97-1.20, 0.140	1.07, 0.96-1.20, 0.193
Negative	1.08, 1.05-1.12, <0.001	1.08, 1.04-1.12, <0.001
TNM stage		
I-II	1.50, 1.24-1.81, <0.001	1.36, 1.08-1.70, 0.009
III-IV	1.06, 1.03-1.09, <0.001	1.06, 1.03-1.10, <0.001
The Lauren's classification		
Diffuse type	1.19, 1.14-1.25, <0.001	1.18, 1.12-1.25, <0.001
Intestinal type	1.06, 1.01-1.11, 0.011	1.06, 1.01-1.12, 0.026
Embolus		
Positive	1.20, 1.13-1.28, <0.001	1.19, 1.11-1.28, <0.001
Negative	1.08, 1.04-1.12, <0.001	1.08, 1.04-1.12, <0.001
Tumor size		
≤ 4.5 cm	1.21, 1.12-1.30, <0.001	1.17, 1.07-1.28, <0.001
> 4.5 cm	1.05, 1.02-1.09, 0.003	1.06, 1.02-1.10, 0.002

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval. The effect-size estimates were calculated under the Weibull proportional hazards regression models. *P was adjusted for age, gender, body mass index, smoking, drinking and family cancer history.

Some limitations should be considered when interpreting the results. First, our findings of this prospective cohort study can only be applied to the

type of population enrolled in this study, i.e., postoperative patients for gastric cancer. Second, the maximal follow-up period in this study was 15 years and the minimal period was 5 years, and the recruitment phase lasted across 10 years, while might introduce a possible selection bias. Third, data on *Helicobacter pylori* infection are not available for us, and *Helicobacter pylori* infection has been proven to play an important role in gastric carcinogenesis. In addition, data on patients' nutritional conditions are also not available for us. So the residual confounding roles of *Helicobacter pylori* infection and nutritional status in the prognosis of preoperative NLR for gastric cancer mortality cannot be excluded. Fourth, all study patients were consecutively enrolled from a mono-center (Fujian Provincial Cancer Hospital) between early 2000 and late 2010, and during this 10-year period remarkable advances in surgical techniques might introduce a possible bias, which might underestimate the impact of blood-routine parameters on gastric cancer mortality.

Taken together, our findings not only consolidated the prognostic value of preoperative NLR and demonstrated that elevated NLR can predict poor survival in patients with early stage gastric cancer receiving radical gastrectomy, but also optimized the discrimination accuracy of NLR by recommending cutoff values in patients with TNM stage I and II. However, we agree that these cutoff values are tentative and may form the basis of a well-designed prospective study to test their validity. For practical reasons, our findings may provide the basis for future personalized medicine whereby gastric cancer patients with altered preoperative blood-routine parameters who are prone to experience a poor survival experience can be detected early and receive targeted therapeutic interventions.

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Contributors

W.N., X.Z., F.P. drafted the protocol; D.H., H.Z., X.L., G.C., B.L., Y.C., Z.C. obtained statutory and ethics approvals; D.H., H.Z., X.L., C.L. B.L., G.C., Y.C., Z.C. contributed to data acquisition; D.H., F.P., X.Z., W.N. had access to all raw data; D.H., H.Z., W.N. did the data preparation, quality control and analyses, and checked the results; W.N., D.H. drafted the report. All authors contributed to writing the final report and approved the version to be published.

Competing Interests

The authors have declared that no competing interest exists.

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