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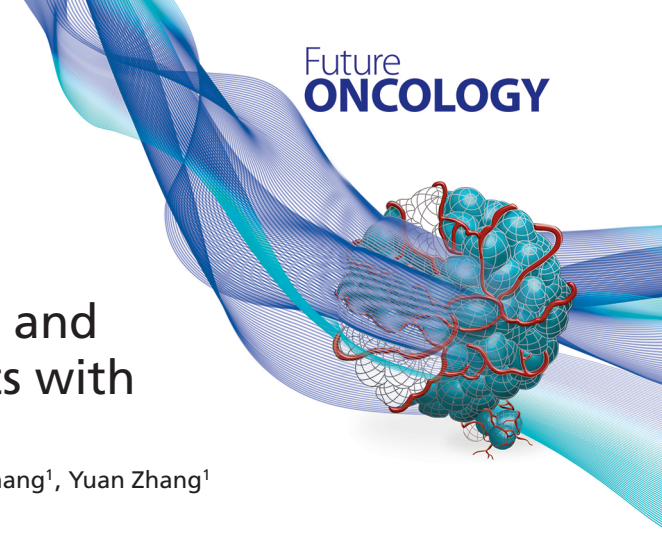

A predictive nomogram developed and validated for gastric cancer patients with triple-negative tumor markers

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Aim: To predict the prognosis of gastric cancer patients with triple-negative tumor markers. **Materials & methods:** Prognostic factors of the nomogram were identified through univariate and multivariate Cox regression analyses. Calibration and receiver operating characteristic curves were used to assess accuracy. Decision curve analysis and concordance indexes were utilized to compare the nomogram with the pathological tumor, node, metastasis stage. **Results:** A nomogram incorporating log odds of positive lymph nodes, tumor size and lymphocyte-to-monocyte ratio was constructed. The calibration and receiver operating characteristic curves (area under the curve >0.85) showed high accuracy in predicting overall survival. The concordance indexes (0.832 vs 0.760; $p < 0.001$) and decision curve analysis demonstrated that the nomogram was superior to the pathological tumor, node, metastasis stage. **Conclusion:** A prediction and risk stratification nomogram has been developed and validated for gastric cancer patients with triple-negative tumor markers.

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Keywords: gastric cancer • log odds of positive lymph nodes • lymphocyte-to-monocyte ratio • nomogram • prognosis • tumor marker

According to the latest epidemiological report, gastric cancer (GC) is now ranked as the fifth most common type of malignant tumor and the fourth leading cause of cancer-related death in 185 countries [1]. GC poses a significant threat to the lives and health of people in eastern Asia, especially China [2]. In 2020, China reported a staggering 479,000 new cases and 374,000 deaths from GC, accounting for 44.0% of the global incidence and 48.6% of global deaths [3]. There are many risk factors contributing to this phenomenon, including high rates of *Helicobacter pylori* infection, unhealthy lifestyles and specific genetic variants [4–6]. Central and Eastern Europe and South America are also high-risk regions for GC [7]. In addition, the incidence of gastric cardia adenocarcinoma has increased markedly in the USA and Northern Europe over recent decades, which may be due to obesity [8].

Radical gastrectomy combined with adjuvant chemotherapy is the current recommended treatment for most GC patients [9,10]. For locally advanced resectable GC patients, neoadjuvant chemotherapy can be considered [11]. However, both surgery and chemotherapy can induce numerous adverse reactions, resulting in a range of physical and psychological symptoms [12]. At the same time, the clinical stages of GC can affect the survival and quality of life of patients. Patients with early GC typically have favorable outcomes after endoscopic resection and do not need chemotherapy. However, due to the insidious onset of GC, most patients with GC are diagnosed at advanced stages, whose prognosis is significantly worse than those with early GC. Patients with advanced GC have many complications, including intestinal obstruction, liver metastasis, ascites, jaundice and hypoproteinemia, and may be not suitable for surgery. In addition, the high invasiveness of advanced GC can result in limited effectiveness of chemotherapy and radiation therapy. The 5-year survival rate for patients with advanced GC is less than 20%, with a median survival time of less than 1 year [13]. Dyspepsia is the most common symptom in early and advanced GC and can be mistakenly attributed to other benign gastrointestinal diseases [14]. Lack of routine endoscopy and the younger age of some patients also contribute to missed early detection of GC [15]. Therefore, there is a crucial need

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for methods that can accurately predict the prognosis of patients with GC. This will enable the implementation of safe, efficient and cost-effective tertiary prevention strategies.

A nomogram is a visualized chart model that is widely used in clinical cancer prediction and considered an effective forecasting tool [16]. In clinical practice, clinicians can utilize a published nomogram based on various patient-specific characteristics to assess the probability of an event, such as death or recurrence, in individual patients. This valuable tool aids in formulating appropriate treatment plans. For instance, patients identified as high risk by a nomogram may be recommended for more frequent community follow-up and additional examinations, such as ctDNA and DNA sequencing [17]. Thus, recurrence or metastasis can be detected earlier and appropriate targeted therapy can be provided. These patients should also be encouraged to participate in clinical trials that may improve prognosis. Furthermore, utilization of a nomogram can aid patients in gaining a clear understanding of their own disease status, indirectly enhancing their quality of life by influencing their lifestyle.

Many tumor markers (TM), inflammatory indicators, immunohistochemistry indexes and small molecules have been developed and validated to economically identify GC patients with poor prognosis [18–20]. These advancements have paved the way for more precise and personalized treatment strategies, with some of these markers already being implemented in clinical practice. However, there is still no single specific biomarker that can provide accurate prognostic information of GC patients to clinicians. The combination of multiple TMs should be one of the optimal schemes. The Task Force of the Japanese Gastric Cancer Association recognized the TMs carcinoembryonic antigen (CEA), CA19-9 and CA72-4 as valuable for detecting recurrence and distant metastasis, predicting patient survival and monitoring after surgery [21]. The combination of CEA, CA19-9 and CA72-4 also demonstrated enhanced efficacy in staging GC and predicting patient survival [18,22]. Over the past decade, these three markers have become standard routine examination items for GC patients in many hospitals. However, with the passage of time, the clinical utilization of CEA, CA19-9 and CA72-4 in predicting GC prognosis revealed certain limitations. One of the primary drawbacks observed was the relatively low overall positive rates associated with each individual marker. For example, in a study conducted by Liu *et al.*, the preoperative serum positive rates of CEA, CA19-9 and CA72-4 were 9.5, 17.9 and 21.5%, respectively [23]. Several other studies also mentioned the low positive rates of CEA, CA19-9 and CA72-4 [24–26]. A meta-analysis assessed the clinical data of GC patients in 46 articles and found that the overall positive rate of these three TMs was less than 30% (24.0% for CEA, 27.0% for CA19-9 and 29.9% for CA72-4) [18]. This phenomenon implied the existence of a large GC population who tested negative for CEA, CA19-9 and CA72-4 simultaneously. We defined this population as GC patients with triple-negative TMs (TNTM). Although it is well known that patients with positive TMs have a worse prognosis, considering the overall low survival rates of GC patients, it is clear that there is also a proportion of patients with poor prognosis in the TNTM population who should not be ignored. Currently, there are no studies available describing the clinical characteristics of these patients or prognostic models to assist in their screening.

Hence, we aimed to utilize clinical and pathological parameters along with inflammatory indicators and TMs, which were reassessed with revised cutoff values, to identify patients with poor prognosis in the TNTM population. By identifying prognostic factors specific to this subgroup, the likelihood of detecting patients with poor prognosis would be increased, indirectly addressing the issue of low positive rates of CEA, CA19-9 and CA72-4. Moreover, we systematically and comprehensively developed a predictive nomogram specifically for GC patients with TNTMs. Our graphical model was expected to assist clinicians in assessing the probability of survival in individual GC patients with TNTMs and detecting high-risk patients based on their total points. Subsequently, high-risk patients would receive personalized treatment, which could improve their prognosis. Our ultimate goal was to improve the survival time and quality of life of patients with GC.

Materials & methods

Patients & data collection

In this retrospective analysis, we first included a total of 896 GC patients who had been pathologically diagnosed at Shanghai General Hospital between January 2012 and December 2019. Figure 1 displays the flowchart illustrating the inclusion and exclusion criteria for the patients. Inclusion criteria for the initial large sample were as follows: patients had received R0 radical gastrectomy without any preoperative treatment, patients were aged 18 years or older, postoperative pathology confirmed GC, no other primary tumors were present and any other non-neoplastic disease that might have been present was not in the acute phase. Patients who met the following exclusion criteria were excluded from the study: missing survival information (including survival months and survival status; n = 128), which was the source of our clinical outcomes; absence of any important TMs (CEA,

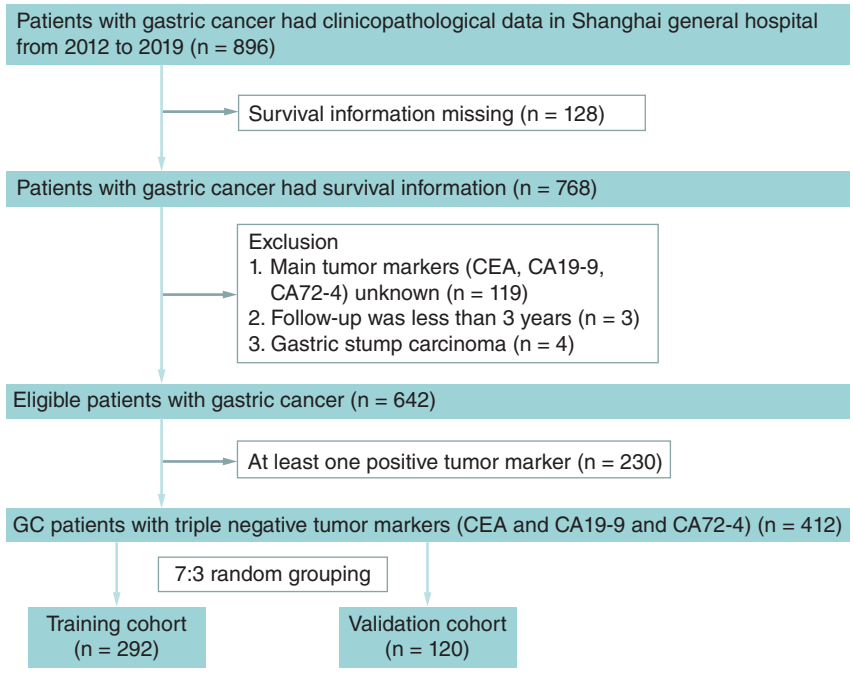

Figure 1. Flowchart of study sample selection.
CEA: Carcinoembryonic antigen; GC: Gastric cancer.

CA19-9 or CA72-4; n = 119), which were key factors for grouping; follow-up time less than 3 years (n = 3); and diagnosis of gastric stump carcinoma (n = 4). The inclusion and exclusion criteria ensured that all participants had similar clinical characteristics and backgrounds, thus ensuring comparability, internal validity and external applicability of the study results. The study was conducted in compliance with the Declaration of Helsinki and was approved by the clinical research ethics committee of Shanghai General Hospital (approval No. 2022KY101). Since this study involved the analysis and sharing of anonymized patient data, informed consent was not required. For the protection of minors, we did not include patients younger than 18 years old.

All data, with the exception of survival information, were extracted from electronic medical records and independently verified by three authors. Survival information was collected through telephone follow-up. The data collection comprised basic demographic information (sex and age), routine blood tests (neutrophil count, lymphocyte count, monocyte count, platelet count, CEA, CA19-9 and CA72-4 levels), clinicopathological data (tumor size; tumor location; tumor depth; lymph node involvement; log odds of positive lymph nodes [LODDS]; pathological tumor, node, metastasis [pTNM] stage and Ki-67) and follow-up information (survival time and survival status). The results of blood tests were recorded 1 week before operation. Serum levels of CEA, CA19-9 and CA72-4 were measured using an electrochemiluminescence immunoassay, conducted by Cobas (Roche, Germany), which is a widely utilized molecular detection method in clinical practice [27]. Systemic inflammatory markers were calculated using the following formulas: the neutrophil-to-lymphocyte ratio (NLR) was determined by dividing the absolute neutrophil count by the absolute lymphocyte count, the platelet-to-lymphocyte ratio (PLR) was determined by dividing the absolute platelet count by the absolute lymphocyte count, the lymphocyte-to-monocyte ratio (LMR) was determined by dividing the absolute lymphocyte count by the absolute monocyte count and the systemic immune–inflammation index (SII) was determined by multiplying the platelet count and the neutrophil count and then dividing by the lymphocyte count. Follow-up was conducted by reviewing medical records and making phone calls. The pTNM stage was determined based on the eighth edition American Joint Committee on Cancer (AJCC) tumor, node, metastasis classification. The first follow-up was 1 month after surgery. Thereafter, patients were generally monitored at 3-month intervals during the first 2 years and then every 6 months (3–5 years). Patients with locally advanced GC received adjuvant chemotherapy during the follow-up period. Overall survival (OS) was calculated as the time from the initial surgery to either the date of death or the last follow-up. The follow-up period concluded in January 2023.

Construction & validation of the nomogram

Enrolled GC patients with TNTMs were identified and randomly allocated into a training set consisting of 292 patients and a validation set consisting of 120 patients at a ratio of 7:3. To ensure that outcome events were evenly distributed between the two cohorts, the R function 'createDataPartition' was utilized in this step for implementation. In the training set, univariate Cox regression analyses were used to derive hazard ratios (HR), CIs and p-values for 16 characteristics. All 16 characteristics were determined based on data availability and clinical evidence rather than on statistical significance [28]. Only variables with $p<0.05$ in the univariate analysis were included in the following multivariate analysis. Subsequently, independent prognostic factors ($p<0.05$) used to construct the nomogram were identified through multivariate Cox regression analysis. The optimal cutoff values for each continuous variable were obtained through X-tile (https://medicine.yale.edu/lab/rimm/research/software/) [29]. Based on the significant factors, the prognostic nomogram was constructed using R 4.3.1 software (www.r-project.org/).

Based on articles regarding nomogram and survival analysis methodology [30,31], multiple approaches were employed to confirm the feasibility and reliability of our nomogram. First, the calibration plots of the nomogram were compared with the standard curves to verify the consistency of the nomogram. Second, the discriminating ability of the nomogram was assessed by the area under the curve (AUC), where a value greater than 0.85 indicated good performance of the model. Additionally, the prognostic value of the nomogram was compared with the pTNM stage through analysis of the concordance index (C-index) and decision curve analysis (DCA). When the C-index was higher than 0.7, the model could be considered to have good predictive performance. The validity of the nomogram's risk stratification was confirmed by Kaplan–Meier survival analysis [32].

Definition of LODDS

LODDS was calculated as the loge – (number of positive lymph nodes + 0.5) / (number of negative lymph nodes + 0.5) – which quantified the relationship between positive and negative lymph nodes in cases in which lymph nodes were retrieved [33]. For the entire group, the optimal cutoff values of LODDS were determined by X-tile to be -1.1 and -0.1. In both the training and validation cohorts, LODDS was divided into three intervals: ≤-0.9, -0.9 to -0.2 and >-0.2.

Statistical analysis

Kaplan–Meier survival analysis, χ^2 test and univariate and multivariate Cox regression analyses were performed using SPSS Statistics 26.0 (IBM Corporation, NY, USA) for Windows (Microsoft Corporation, WA, USA). Kaplan–Meier survival curves were generated using an online drawing tool (www.xiantaozi.com/). All steps of nomogram construction and validation were implemented using R software. The nomogram and its calibration plots were generated using the R package 'rms', the C-indexes were calculated using the R package 'Hmisc', the receiver operating characteristic curves were plotted by the R package 'timeROC' and DCA was performed through 'dcurves'. The significance level for all tests was set at 0.05 on a two-sided basis.

Results

Demographic & clinical characteristics of GC patients

Based on the inclusion and exclusion criteria, data for a total of 642 GC patients were collected and analyzed (Figure 1). In our study, we defined patients with preoperative serum CEA, CA19-9 and CA72-4 levels simultaneously lower than the normal reference values as GC patients with TNTMs. Moreover, GC patients with at least one positive TM (positive TMs >0) were defined as patients with non-TNTMs. Based on the reference values of TMs (CEA <5 ng/ml, CA19-9 <37 U/ml and CA72-4 <6.9 U/ml) in our hospital, eligible GC patients were categorized into two groups: 230 (35.8%) with non-TNTMs and 412 (64.2%) with TNTMs (Supplementary Figure 1). The positive rate was 18.2% (117 of 642) for CEA, 13.7% (88 of 642) for CA19-9 and 17.1% (110 of 642) for CA72-4. More detailed characteristics are displayed in Table 1.

Prognostic value of CEA, CA19-9 & CA72-4

In the entire group, the 1-, 3- and 5-year OS rates were 91.3, 77.2 and 66.1%, respectively. The median follow-up time was 45.6 months. According to the results of Kaplan–Meier survival curves and log-rank tests, patients with positive CEA, CA19-9 or CA72-4 alone exhibited a significantly poorer prognosis ($p < 0.001$; $p < 0.001$ and $p = 0.001$, respectively; Figure 2A–C). The difference in estimated 3-year OS rates between positive and negative TMs was 59.9 versus 80.9% for CEA, 60.2 versus 79.9% for CA19-9 and 67.9 versus 79.2% for CA72-4. The

Table 1. Demographic and clinical characteristics of gastric cancer patients.

Variable	Patients
Total, n (%)	642 (100.0)
Sex, n (%)	
Male	427 (66.5)
Female	215 (33.5)
Age, years, n (%)	
<65	271 (42.2)
≥65	371 (57.8)
Tumor size, cm, n (%)	
<5	400 (62.3)
≥5	242 (37.7)
Tumor location, n (%)	
Proximal	134 (20.9)
Distal	319 (49.7)
Body	189 (29.4)
T stage, n (%)	
1	173 (26.9)
2	88 (13.7)
3	200 (31.2)
4	181 (28.2)
N stage, n (%)	
0	265 (41.3)
1	99 (15.4)
2	105 (16.4)
3	173 (26.9)
LODDS, median (IQR)	-1.100 (-1.653 to 0.351)
pTNM, n (%)	
I	205 (31.9)
II	156 (24.3)
III	280 (43.6)
IV	1 (0.2)
Nerve invasion, n (%)	
Negative	321 (50.0)
Positive	289 (45.0)
Not available	32 (5.0)
Vascular invasion, n (%)	
Negative	276 (43.0)
Positive	324 (50.5)
Not available	42 (6.5)
Ki-67, %, n (%)	
≤25	37 (5.8)
≤50	173 (26.9)
≤75	224 (34.9)
≤100	176 (27.4)
Not available	32 (5.0)

CEA: Carcinoembryonic antigen; IQR: Interquartile range; LMR: Lymphocyte-to-monocyte ratio; LODDS: Log odds of positive lymph nodes; N: Node; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; pTNM: Pathological tumor, node, metastasis stage; SII: Systemic immune–inflammation index; T: Tumor; TM: Tumor marker; TNTMs: Triple-negative tumor markers.

Table 1. Demographic and clinical characteristics of gastric cancer patients (cont.).

Variable	Patients
CEA, ng/ml, n (%)	
<5	525 (81.8)
≥5	117 (18.2)
CA19-9, U/ml, n (%)	
<37	554 (86.3)
≥37	88 (13.7)
CA72-4, U/ml, n (%)	
<6.9	532 (82.9)
≥6.9	110 (17.1)
TM status, n (%)	
TNTMs	412 (64.2)
Non-TNTMs	230 (55.8)
Systemic inflammatory markers, median (IQR)	
NLR	2.15 (1.59–2.93)
PLR	131.43 (99.16–177.77)
LMR	4.73 (3.55–6.13)
SII	456.00 (306.85–704.88)

CEA: Carcinoembryonic antigen; IQR: Interquartile range; LMR: Lymphocyte-to-monocyte ratio; LODDS: Log odds of positive lymph nodes; N: Node; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; pTNM: Pathological tumor, node, metastasis stage; SII: Systemic immune–inflammation index; T: Tumor; TM: Tumor marker; TNTMs: Triple-negative tumor markers.

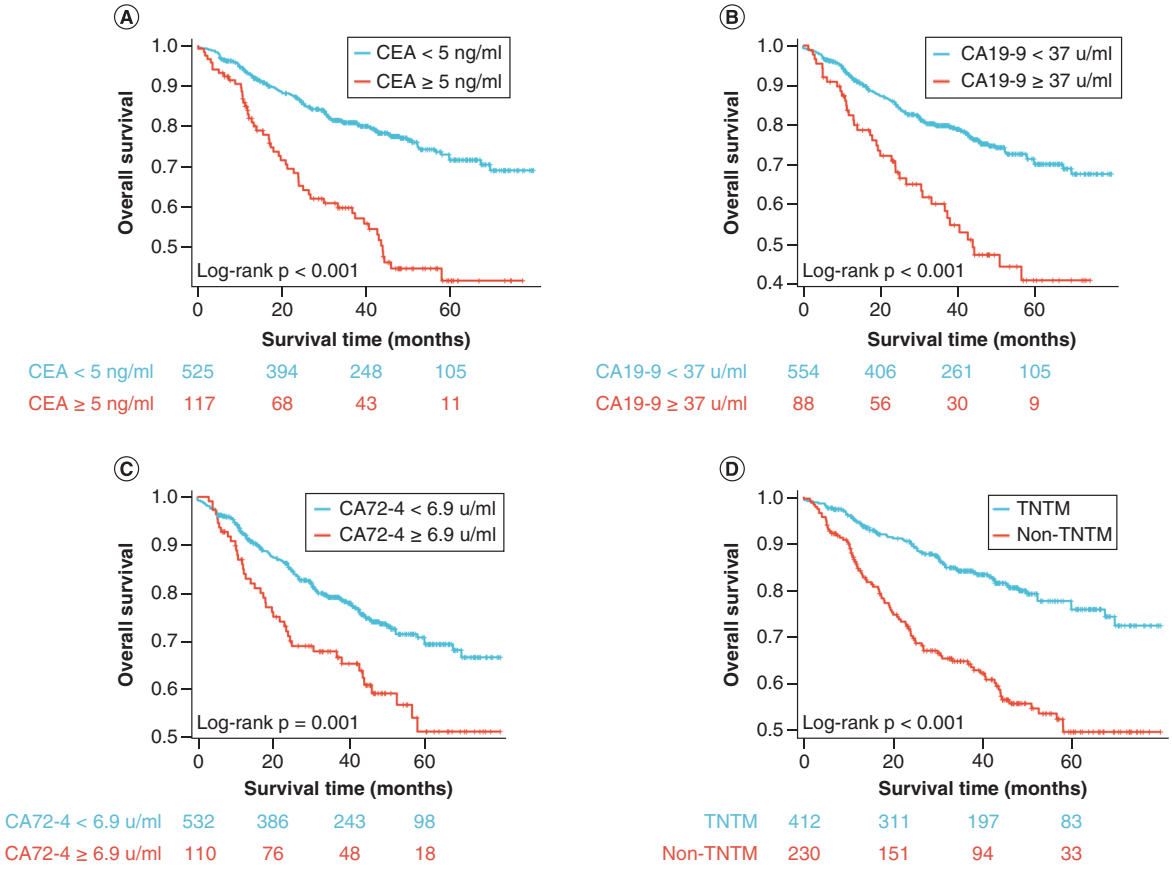

Figure 2. Survival analyses of tumor markers in the entire group. (A–D) Kaplan–Meier survival analyses of overall survival for all gastric cancer patients according to **(A)** CEA, **(B)** CA19-9, **(C)** CA72-4 and **(D)** tumor marker status. CEA: Carcinoembryonic antigen; TNTM: Triple-negative tumor marker.

estimated 5-year OS rates in the positive CEA, CA19-9 and CA72-4 groups were 41.9, 41.1 and 51.3%, respectively, compared with 71.7, 70.2 and 69.4%, respectively, in the corresponding negative groups. Positive preoperative TMs often indicate a heavier tumor burden or more active cancer cells [34]. This would explain why the survival curves of the positive and negative groups were significantly different.

Next, other clinicopathological characteristics were analyzed by Cox regression to determine whether these three TMs were valuable prognostic risk factors. The optimal cutoff values of LODDS, NLR, PLR, LMR and SII were determined by X-tile, which analyzed data from a total of 642 GC patients (Supplementary Figure 2). Univariate analysis revealed that age, tumor size, tumor stage, node stage (N stage), LODDS, pTNM, CEA, CA19-9, CA72-4, TM status, NLR, PLR, LMR and SII were related to OS ($p < 0.05$; Supplementary Table 1). The HRs of CEA, CA19-9 and CA72-4 were 2.667 (95% CI: 1.920–3.703), 2.441 (95% CI: 1.707–3.489) and 1.761 (95% CI: 1.240–2.502), respectively. When the three TMs were included independently in the multivariate Cox analysis, CEA (HR = 1.905; 95% CI: 1.363–2.662) was an independent risk factor for OS, in addition to age (HR = 1.465; 95% CI: 1.032–2.080), tumor size (HR = 1.637; 95% CI: 1.169–2.291), LODDS (-1.1<LODDS≤-0.1: HR = 3.369; 95% CI: 1.850–6.136; LODDS>-0.1: HR = 6.880; 95% CI: 3.586–13.199), pTNM stage (stage II: HR = 1.172; 95% CI: 0.526–2.610; stage III: HR = 2.148; 95% CI: 0.951–4.853; stage IV: HR = 4.259; 95% CI: 0.475–38.179) and NLR (HR = 1.617; 95% CI: 1.096–2.384).

We concluded that CEA, CA19-9 and CA72-4 were useful for prognostic evaluation of GC patients but that their low positive rates inevitably missed some patients with poor prognosis. Therefore, we attempted to construct a novel nomogram to differentiate patients with poor prognosis from those with TNTMs.

Characteristics of GC patients with TNTMs

In this study, CEA, CA19-9 and CA72-4 were combined into 'TM status' (i.e., GC patients with TNTMs or non-TNTMs). We observed that survival time in GC patients with non-TNTMs was significantly shorter compared with those with TNTMs (log-rank $p < 0.001$; Figure 2D). When TM status was included in the multivariate Cox analysis instead of a single TM, TM status (HR = 1.505; 95% CI: 1.092–2.074) was one of the independent prognostic factors for OS (Supplementary Table 1). The association between TM status and other characteristics of GC patients with TNTMs is demonstrated in Supplementary Table 2. The results indicated that GC patients with TNTMs exhibited more favorable clinicopathological characteristics, including age, tumor size, tumor stage, N stage, LODDS, pTNM stage, LMR and SII ($p < 0.05$), compared with those with non-TNTMs. Moreover, GC patients with TNTMs demonstrated lower levels of Ki-67 ($p = 0.013$), and their primary tumors tended to originate in the gastric body ($p = 0.006$). This was consistent with the results of most studies [35,36], indicating that the combination of CEA, CA19-9 and CA72-4 is a good non-invasive method for predicting the prognosis of GC patients.

Grouping & classification of variables in GC patients with TNTMs

A total of 412 GC patients with TNTMs were randomly divided into a training set of 292 patients and a validation set of 120 patients at a ratio of 7:3 (Figure 1). All characteristics were required to be not obviously different between the two groups. The demographic and clinicopathological characteristics of the training and validation cohorts are listed in Supplementary Table 3. The χ^2 test was employed to assess the homogeneity of data distribution among groups ($p > 0.05$). As the sample population changed after GC patients with TNTMs were isolated for analysis, X-tile was utilized to determine a novel cut-off point for classifying continuous variables. By conducting Kaplan–Meier analyses and calculating p-values at each cut-off point, X-tile selects one or more optimal cut-off points, enabling researchers to predict patient outcomes more effectively [29]. To investigate the significance of reusing TMs in GC patients with TNTMs, we obtained the respective novel cut-off points of CEA, CA19-9 and CA72-4 based on their original parameters (Supplementary Figure 3). Interestingly, the cut-off points of LMR (5.0 vs 5.0) and tumor size (5.0 vs 4.9 cm) remained consistent across the entire GC patient population as well as GC patients with TNTMs, indicating the reliability and stability of these two predictors as prognostic factors in these specific groups.

Univariate & multivariate Cox analyses of the training cohort

In the training cohort, univariate Cox analysis showed that sex, age, tumor size, tumor stage, N stage, LODDS, pTNM stage, NLR and LMR were associated with OS ($p < 0.05$; Table 2). However, it appeared that all three TMs lost predictive value in GC patients with TNTMs, even after redefining the cutoff values. In addition, LMR

Table 2. Univariate and multivariate Cox regression analyses of variables in the training cohort.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Sex						
Male	–	1	–			
Female	0.508	0.271–0.951	0.034			
Age, years						
<76	–	1	–			
≥76	2.534	1.435–4.476	0.001			
Tumor size, cm						
<4.9	–	1	–	–	1	–
≥4.9	3.504	2.038–6.025	< 0.001	2.460	1.414–4.280	0.001
Tumor location			0.251			
Proximal	–	1	–			
Distal	0.554	0.273–1.122	0.101			
Body	0.630	0.305–1.302	0.212			
T stage			< 0.001			
1	–	1	–			
2	2.498	0.805–7.75	0.113			
3	2.170	0.788–5.972	0.134			
4	10.572	4.403–25.384	< 0.001			
N stage			< 0.001			
0	–	1	–			
1	0.360	0.046–2.846	0.360			
2	4.660	1.963–11.062	< 0.001			
3	13.602	6.436–28.745	< 0.001			
LODDS			< 0.001			< 0.001
≤-0.9	–	1	–	–	1	–
>-0.9, ≤-0.2	6.866	3.343–14.105	< 0.001	5.161	2.482–10.733	< 0.001
>-0.2	25.154	11.613–54.481	< 0.001	19.986	9.026–44.254	< 0.001
pTNM			< 0.001			
I	–	1	–			
II	2.047	0.710–5.902	0.185			
III	10.349	4.371–24.505	< 0.001			
Ki-67, %						
≤50	–	1	–			
>50	0.793	0.450–1.398	0.423			
CEA, novel cutoff, ng/ml						
<1.1	–	1	–			
≥1.1	1.541	0.752–3.158	0.237			
CA19-9, novel cutoff, U/ml						
<3.6	–	1	–			
≥3.6	1.412	0.509–3.914	0.508			
CA72-4, novel cutoff, U/ml						
<0.9	–	1	–			
≥0.9	2.340	0.729–7.511	0.153			
NLR						
<1.8	–	1	–			
≥1.8	2.011	1.090–3.712	0.025			

CEA: Carcinoembryonic antigen; HR: Hazard ratio; LMR: Lymphocyte-to-monocyte ratio; LODDS: Log odds of positive lymph nodes; N: Node; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; pTNM: Pathological tumor, node, metastasis stage; SII: Systemic immune–inflammation index; T: Tumor.

Table 2. Univariate and multivariate Cox regression analyses of variables in the training cohort (cont.).

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
PLR						
<190.2	–	1	–			
≥190.2	1.639	0.901–2.980	0.106			
LMR						
<5.0	–	1	–	–	1	–
≥5.0	0.337	0.180–0.632	0.001	0.491	0.260–0.926	0.028
SII						
<711.8	–	1	–			
≥711.8	1.681	0.924–3.059	0.089			

CEA: Carcinoembryonic antigen; HR: Hazard ratio; LMR: Lymphocyte-to-monocyte ratio; LODDS: Log odds of positive lymph nodes; N: Node; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; pTNM: Pathological tumor, node, metastasis stage; SII: Systemic immune–inflammation index; T: Tumor.

(HR = 0.337; 95% CI: 0.180–0.632) acted as the only protective factor for OS among the systemic inflammatory markers. Multivariate Cox analysis demonstrated that LODDS (-0.9<LODDS≤-0.2: HR = 5.161; 95% CI: 2.482–10.733; LODDS>-0.2: HR = 19.986; 95% CI: 9.026–44.254), tumor size (HR = 2.460; 95% CI: 1.414–4.280) and LMR (HR = 0.491; 95% CI: 0.260–0.926) were independent risk factors for OS in GC patients with TNTMs ($p < 0.001$; $p = 0.001$ and $p = 0.028$, respectively).

Construction & validation of novel nomogram

Based on the findings of multivariate Cox analysis, we integrated LODDS, tumor size and LMR to develop an individualized nomogram for predicting the survival probability of GC patients with TNTMs (Figure 3A). The calibration plots demonstrated strong agreement between the observed and predicted OS outcomes (Figure 3B–D). In our developed nomogram, LODDS exerted the most significant impact on the prognosis of GC patients with TNTMs followed by tumor size, with LMR being identified as a protective factor.

We utilized receiver operating characteristic curves to assess the predictive accuracy of our nomogram model for prognosis in GC patients with TNTMs (Figure 4A–C). In the training cohort, the AUCs for 1-, 3- and 5-year predictions were 0.870, 0.880 and 0.862, respectively. In the validation cohort, the AUCs at 1, 3 and 5 years were 0.945, 0.845 and 0.896, respectively. Furthermore, in the whole cohort, the AUCs for 1-, 3- and 5-year predictions were 0.872, 0.862 and 0.877, respectively. The closer the AUC value was to 1, the stronger the predictive power of the model was. The majority of AUC values exceeded 0.85, indicating that the nomogram demonstrated favorable performance in predicting OS in GC patients with TNTMs.

Harrell's C-index was used to measure the accuracy of the survival analysis model in ordering risk among individuals. To assess whether the nomogram exhibited superior efficacy in predicting OS compared with the traditional pTNM stage, we compared C-indexes among the training cohort, validation cohort and whole cohort. The C-indexes of the nomogram in the training cohort, validation cohort and whole cohort were significantly higher than those of the pTNM stage (0.835, 0.837 and 0.832 vs 0.750, 0.791 and 0.760, respectively; $p < 0.05$), which indicated that the nomogram could predict the OS of GC patients with TNTMs more accurately than the pTNM stage (Table 3). In addition, DCA was used to assess the utility of predictive models in clinical decision-making. At the same probability threshold, DCA of the training cohort, validation cohort and whole cohort showed that the novel nomogram often had higher net benefit than the pTNM stage (Figure 4D–F). As a result, for our samples, the new nomogram was not inferior to the traditional pTNM stage for clinical decision-making and showed potential for clinical application.

Risk stratification based on the nomogram

To evaluate the potential of our innovative nomogram for improving the risk stratification of GC patients with TNTMs, we collected the total points assigned to each participant and conducted Kaplan–Meier survival analysis. Based on the distribution of total points in the training cohort (Figure 5A), we identified two intervals with approximately equal numbers of patients and defined them as the low-risk group (0–24.20) and the high-risk group (30.89–155.09). This division would help reduce within-group differences and confounding effects [37,38]. The

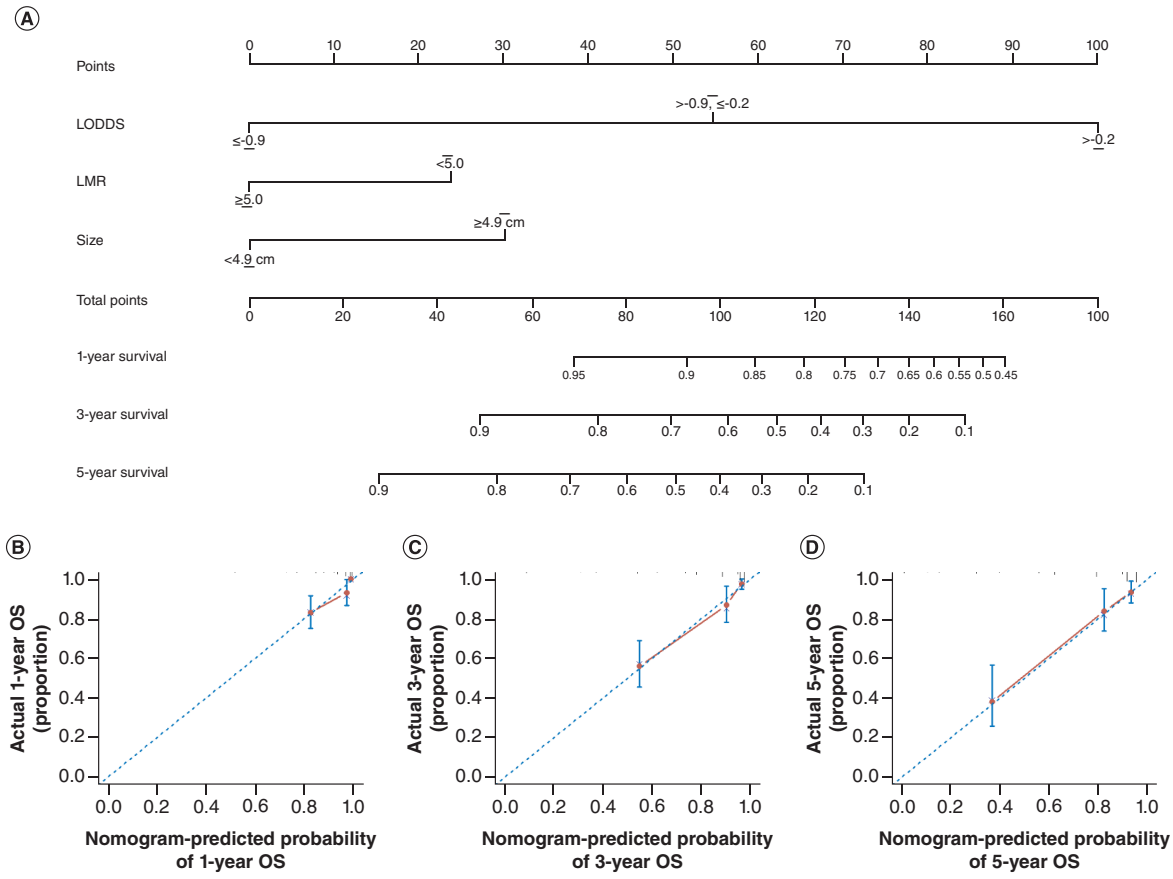

Figure 3. Nomogram and its calibration curves. (A) Novel nomogram for predicting 1-, 3- and 5-year OS in gastric cancer patients with triple-negative tumor markers. **(B–D)** Corresponding calibration curves.
LMR: Lymphocyte-to-monocyte ratio; LODDS: Log odds of positive lymph nodes; OS: Overall survival.

low-risk group (0–24.20) had significantly better OS than the high-risk group (30.89–155.09) regardless of cohort (log-rank $p < 0.001$; Figure 5B–D). Therefore, the nomogram demonstrated good performance in dividing GC patients with TNTMs into two subgroups with different prognoses.

Discussion

It has been widely demonstrated that preoperative positive CEA, CA19-9 and CA72-4 are related to worse outcome and recurrence after surgery in GC [39,40]. However, because of the heterogeneity of GC and the nonspecificity of TMs, abnormal expression of TMs cannot be found in many GC patients through preoperative laboratory tests [41], meaning that some with high risk are mis-predicted as good outcome ones. Usually, combinations of TMs with other clinical factors, such as inflammatory indicators [42], ncRNAs [43,44] and hemoglobin [45], are employed to enhance the detection rate of high-risk GC patients. However, this approach comes with certain limitations. For instance, the newly included clinical factors may also lack specificity, and their reference values are derived from the general population. As a result, the number of false-positive cases inevitably increases. Our study is the first to present a direct analysis of data obtained from GC patients with TNTMs. Our innovative nomogram incorporating LODDS, tumor size and LMR was developed and validated to predict the OS probability of GC patients with TNTMs and displayed good working efficacy and promising clinical applicability. In practice, clinicians can initially categorize GC patients into TNTM and non-TNTM groups based on preoperative serum CEA, CA19-9 and CA72-4 levels. Next, the novel nomogram can be adapted to differentiate high-risk individuals from the TNTM group. Finally, low-risk patients will undergo routine treatment, whereas high-risk patients identified by the nomogram as well as the non-TNTM group will be recommended for more frequent follow-up visits and additional examinations, such as ctDNA and DNA sequencing, to determine further individual treatment. For example, patients with HER2 amplification would undergo treatment with trastuzumab, whereas those with abnormal ctDNA levels would

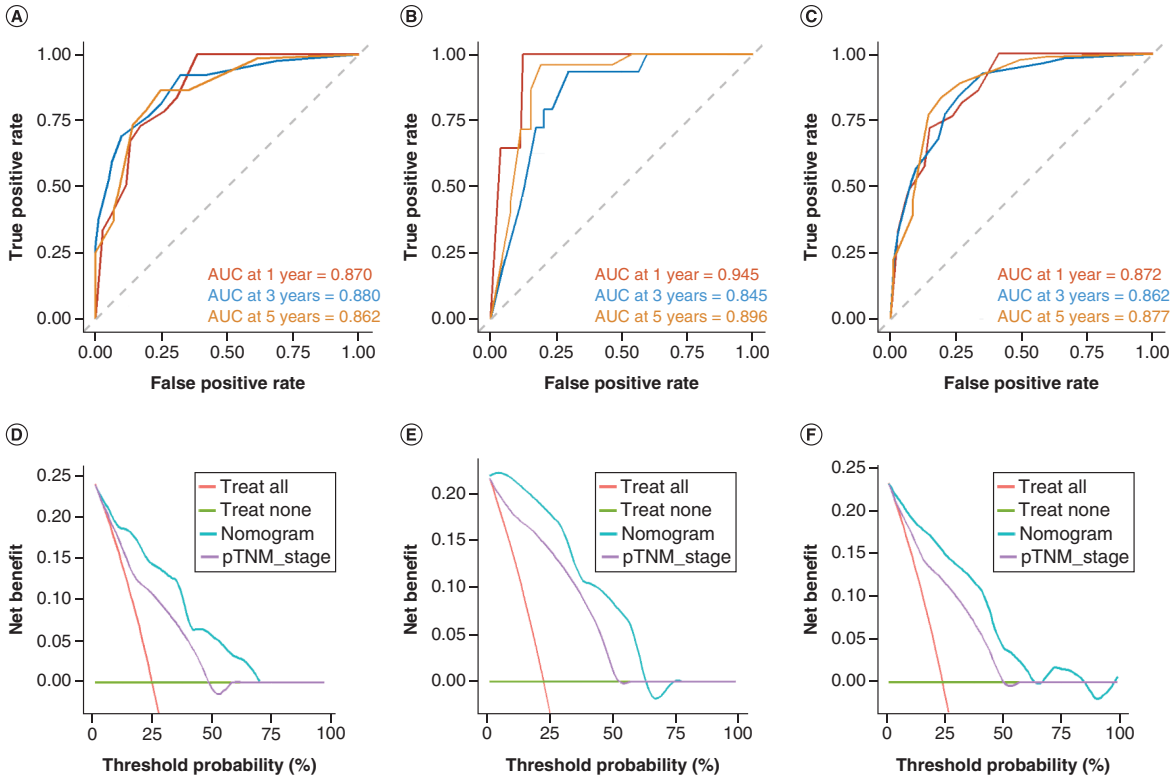

Figure 4. Receiver operating characteristic analysis and decision curve analysis for the nomogram. (A–C) Receiver operating characteristic analysis of the nomogram for 1-, 3- and 5-year overall survival in the **(A)** training cohort, **(B)** validation cohort and **(C)** whole cohort. **(D–F)** Decision curve analysis of the clinical efficacy of the nomogram compared with the eighth edition American Joint Committee on Cancer pTNM classification in the **(D)** training cohort, **(E)** validation cohort and **(F)** whole cohort.
AUC: Area under the curve; pTNM: Pathological tumor, node, metastasis.

Table 3. Comparison of prediction accuracy between the nomogram and pathological tumor, node, metastasis stage.

Variable	C-index	95% CI	p-value
Training cohort			< 0.001
Nomogram	0.835	0.786–0.884	
pTNM stage	0.750	0.689–0.811	
Validation cohort			0.044
Nomogram	0.837	0.747–0.927	
pTNM stage	0.791	0.728–0.854	
Whole cohort			< 0.001
Nomogram	0.832	0.789–0.875	
pTNM stage	0.760	0.712–0.808	

C-index: Concordance index; pTNM: Pathological tumor, node, metastasis.

undergo systemic imaging. Under ideal conditions, GC patients would receive personalized treatment tailored to their specific needs, subsequently improving their OS time and quality of life.

LODDS is a valuable novel prognostic indicator for solid tumors, particularly those prone to lymph node metastasis, such as breast cancer and colorectal cancer [46,47]. A previous study by our group demonstrated that LODDS is an independent prognostic factor in both left- and right-sided colon cancer [48]. Sun *et al.* first proved the prognostic value of LODDS for patients with GC, demonstrating that it was more reliable than pathological node and rN (the ratio of metastatic lymph nodes to total retrieved lymph nodes) classification [49]. A multicenter analysis of 7620 patients in China validated that LODDS was superior to the pathological node stage based on the 8th

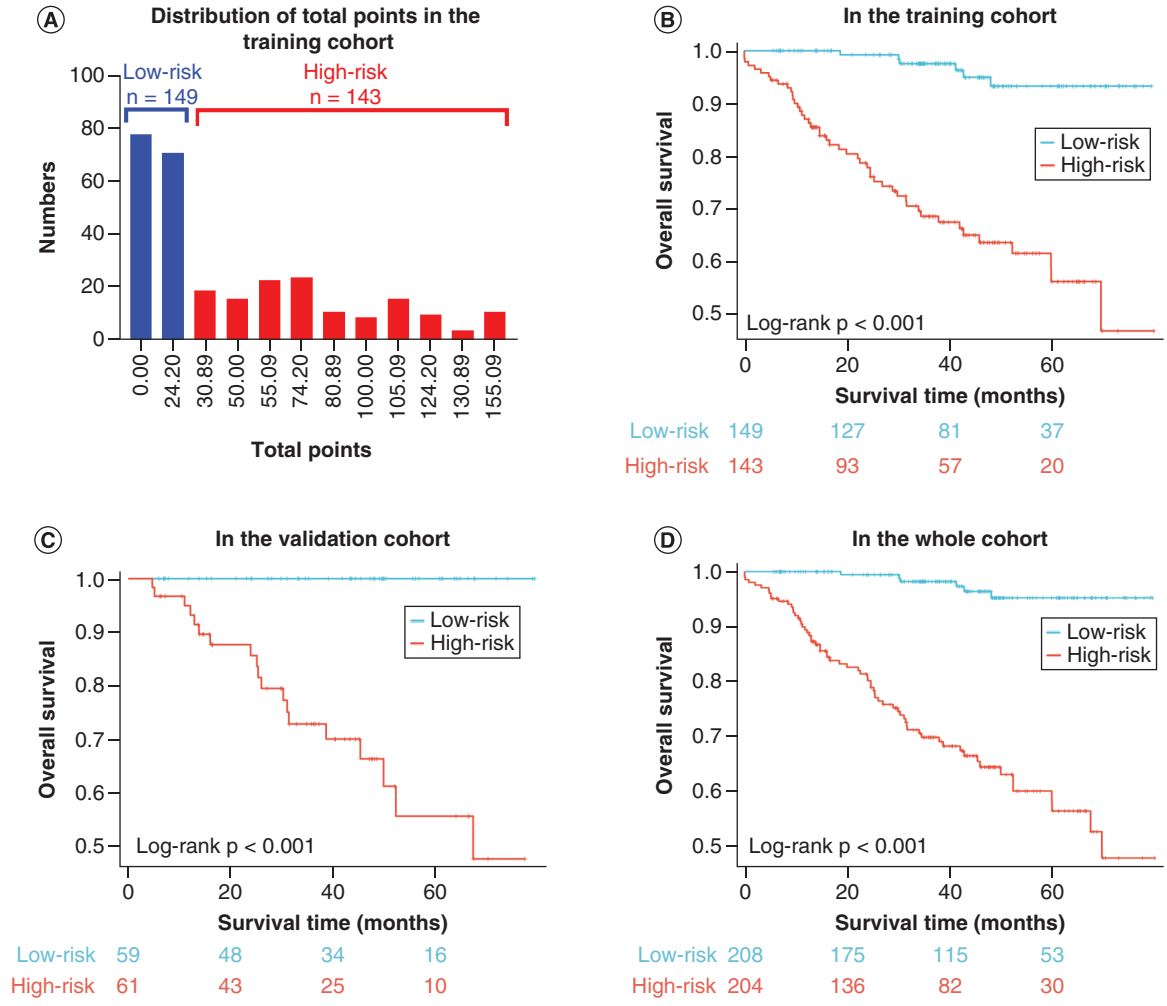

Figure 5. Risk stratification based on the nomogram. (A) Bar graph depicting the distribution of total points in the training cohort. **(B–D)** Kaplan–Meier survival curves of risk groups stratified based on the nomogram for gastric cancer patients with triple-negative tumor markers in the **(B)** training cohort, **(C)** validation cohort and **(D)** whole cohort.

AJCC TNM system for predicting the prognosis of patients undergoing gastrectomy for GC [50]. Interestingly, Liu *et al.* reported that the ratio between metastatic and examined lymph nodes staging was superior to LODDS for evaluating the prognosis of GC [51], but their result demanded more validation. So we still chose LODDS because there were more articles to support its reliability. Based on our study findings, we observed that LODDS had a more significant impact on the assessment of GC prognosis compared with pathological N stage. When both LODDS and N stage were included in the multivariate analysis, LODDS remained statistically significant, whereas N stage lost its significance. Moreover, LODDS was proved to be significantly associated with the OS of GC patients with TNTMs for the first time. Hence, LODDS demonstrated substantial potential for clinical implementation in GC prediction.

Multiple studies have shown that systemic inflammatory markers are associated with increased cancer risk and mortality [52]. Cancer cells produce cancer-related inflammatory mediators that alter the levels of blood cells and influence the development, progression and metastasis of tumors [53]. Unlike other common systemic inflammatory markers, LMR has been consistently confirmed as a favorable factor in various types of tumors, including GC [54]. According to a multicenter study from Japan, low preoperative LMR (HR = 2.271; 95% CI: 1.382–3.734) is an adverse prognostic marker in GC patients [55]. Moreover, a meta-analysis comprising 4908 patients concluded that low pretreatment LMR is significantly associated with decreased OS (HR = 0.66; 95% CI: 0.54–0.82) [56]. In our study, GC patients with high LMR had statistically better OS than those with low LMR, which was consistent

with both the multicenter study and the meta-analysis [55,56]. Based on the results of our multivariate Cox analysis and nomogram, we proved that LMR might be a valuable protective factor for OS in GC patients with TNTMs.

Since serum TM tests are fast, inexpensive and noninvasive, they have become widely available in most hospitals in China. The clinical significance of CEA, CA19-9 and CA72-4 in diagnosing, monitoring and evaluating GC in the Chinese population is clear [57,58]. Still, we attempted to explore the prognostic value of these three TMs in GC patients with TNTMs. Following determination of their optimal cutoff values using X-tile analysis, survival analysis revealed no statistically significant association between these three TMs and OS ($p > 0.05$). In addition, we noted that the novel cutoff values were low, leading to an increase in false-positive rates. CEA, CA19-9 and CA72-4 are likely not suitable for use in predicting prognosis in GC patients with TNTMs.

Although our nomogram exhibited satisfactory predictive performance for OS in GC patients with TNTMs, the current study has several limitations. First, our participants were all from a single healthcare institution, so there was a lack of an external validation set, which limits the generalizability and applicability of our nomogram. Therefore, clinicians should ensure that the characteristics of patients in their clinics are similar to those in this study before applying the nomogram. Second, as the exact time of tumor recurrence after surgery could not be determined for some patients, we did not validate the efficacy of the nomogram in predicting progression-free survival. If a nomogram for predicting progression-free survival in GC patients with TNTMs is to be constructed, the included variables may change. Third, our study was subject to inevitable patient selection bias, which means that the sample selection might not be representative of the entire target population. We minimized selection bias by ensuring an adequate sample size, excluding participants with inadequate follow-up and selecting stable and objective variables. In addition, our institution is a large general hospital, which made the source of our samples more extensive. In the future, we will work with other institutions to further develop and advance the application of the nomogram. Considering that our samples were all from surgical patients, there was a lack of patients with advanced GC with distant metastasis. We will add patients with advanced GC to verify the applicability of the nomogram in different stages of GC. More novel biomarkers, such as serum circRNA and lactate, will also be collected as potential variables.

Conclusion

In summary, our nomogram demonstrated accurate prediction of OS and risk stratification in GC patients with TNTMs following surgery. This was the first study to directly analyze GC patients with TNTMs and provide an alternative auxiliary decision-making model specifically for this subgroup. Our study was looking forward to improving the prognosis evaluation of patients with GC in the perioperative period. Through the formulation of individualized treatment, the survival time and quality of life of these patients will be improved.

Summary points

- Of the 642 gastric cancer (GC) patients in our study, 412 (64.2%) had triple-negative tumor markers (TNTM).
- The combination of carcinoembryonic antigen, CA19-9 and CA72-4 remained a good noninvasive method for predicting the prognosis of GC patients, though the overall positive rates of these three tumor markers were low.
- Three independent prognostic factors (log odds of positive lymph nodes, lymphocyte-to-monocyte ratio and tumor size) were identified in the training cohort of GC patients with TNTMs through multivariate Cox analysis.
- The first predictive nomogram specifically for GC patients with TNTMs was constructed and validated.
- The calibration plots demonstrated strong agreement between the observed and predicted overall survival outcomes.
- In the validation cohort, the areas under the curve of the nomogram at 1, 3 and 5 years were 0.945, 0.845 and 0.896, respectively.
- The concordance indexes and decision curve analysis based on our samples showed that the nomogram was superior to the traditional pathological tumor, node, metastasis stage.
- The nomogram demonstrated good performance in dividing GC patients with TNTMs into low- and high-risk groups.
- The nomogram was helpful in refining the risk stratification of GC patients and formulating individualized treatment.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2023-0626

Author contributions

Study design: C Huang, Y Xu, P Zhang, Z Luo and G Cen. Data collection: Y Xu, P Zhang, Z Luo, S Zhang and Y Zhang. Manuscript preparation: C Huang and Y Xu. Data analysis: Y Xu, P Zhang, Z Luo and G Cen. All authors contributed to the manuscript and approved the submitted version and are responsible for the content.

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Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with an interest in or conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, this study involved the analysis and sharing of anonymized patient data, informed consent was not required.

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