

Article

A Modified NAR Scoring Model Incorporating Immune Infiltration Characteristics to Better Predict Long-Term Survival Following Neoadjuvant Radiotherapy in Rectal Cancer

Xueqing Zhang [†], Yibin Zeng [†], Hui Li, Qingsyang Zhuang, Lirui Tang, Junxin Wu ^{*}  and Jinluan Li ^{*} 

Department of Radiation Oncology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou 350014, China; xueqingzhang21@126.com (X.Z.); zyb9803@163.com (Y.Z.); lihui960815@gmail.com (H.L.); zqy199302@163.com (Q.Z.); tlr330306938@pku.edu.cn (L.T.)

* Correspondence: junxinwufj@aliyun.com (J.W.); lijinluan@fjmu.edu.cn (J.L.)

† These authors contributed equally to this work.

Abstract: (1) Background: The neoadjuvant rectal (NAR) score has been developed as a prognostic tool for survival in locally advanced rectal cancer (LARC). However, the NAR score only incorporates weighted cT, ypT, and ypN categories. This long-term follow-up study aims to modify a novel prognostic scoring model and identify a short-term endpoint for survival. (2) Methods: The prognostic factors for overall survival (OS) were explored through univariate and multivariate analyses. Based on Cox regression modeling, nomogram plots were constructed. Area under the curve (AUC) and concordance indices were used to evaluate the performance of the nomogram. Receiver operating characteristic (ROC) analysis was conducted to compare the efficiency of the nomogram with other prognostic factors. (3) Results: After a long-term follow-up, the 5-year OS was 67.1%. The mean NAR score was 20.4 ± 16.3 . Multivariate analysis indicated that CD8+ T-cell, lymphovascular invasion, and the NAR score were independent predictors of OS. The modified NAR scoring model, incorporating immune infiltration characteristics, exhibited a high C-index of 0.739 for 5-year OS, significantly outperforming any individual factor. Moreover, the predictive value of the nomogram was superior to the AJCC stage and pathological complete regression at 3-year, 5-year, and 10-year time points, respectively. Over time, the model's predictions of long-term survival remained consistent and improved in accuracy. (4) Conclusions: The modified NAR scoring model, incorporating immune infiltration characteristics, demonstrates high accuracy and consistency in predicting OS.

Keywords: rectal cancer; neoadjuvant radiotherapy; prognosis; modified-NAR scoring model; immune infiltration



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1. Introduction

Colorectal cancer is the fourth most commonly diagnosed type of cancer and the second most common cause of cancer-related deaths in the world [1]. Neoadjuvant radiotherapy (nRT) and chemoradiotherapy followed by total mesorectal excision (TME) have become the standard treatment approach for locally advanced rectal cancer (LARC) [2,3]. However, the response to nRT is variable. It can range from pathological complete regression (pCR) to minimal effect or even tumor progression [4]. Additionally, assessing treatment efficacy typically relies on 3- or 5-year survival rates, which can be time-consuming and costly. Therefore, there is a need for early surrogate endpoints in oncology clinical trials to reduce patient numbers and trial duration [5]. Finding a new short-term endpoint for individual prognosis assessment and clinical decision making is essential.

Advancements in treatment have led to the development of various grading systems, including TNM staging, tumor regression grading (TRG), and pCR [6–8]. TNM staging system after nCRT is a key component in the clinical assessment of prognostic prediction and risk stratification, which helps to guide the clinical treatment decisions. Even though

LARC patients with the same TNM staging receive similar treatment their outcomes vary [9]. The TRG system has been developed to evaluate tumor pathologic responses to neoadjuvant therapy [10]. However, there is still no consensus on whether TRG is a prognostic factor for LARC [11]. Only approximately 20% of patients achieved pathologic complete regression (pCR) and had favorable long-term survival [12]. This subset of patients have the opportunity to be treated with a “watch and wait” approach rather than radical surgery [13]. One such endpoint that has been proposed as a prognostic factor for rectal cancer is the neoadjuvant rectal (NAR) score. Valentini’s nomogram for overall survival is used to calculate the NAR score (OS) [14], which incorporates both clinical and pathological characteristics on the T stage and N stage before and after nRT [15]. Patients with low NAR scores have demonstrated favorable tumor outcomes [16] and have been evaluated in the Phase 2 Randomized Clinical Trial [17]. The previous study confirmed the predictive role of the NAR score for DFS and its surrogate at the individual patient level in a large randomized phase III trial. The NAR score may help oncologists to accelerate response-adapted therapeutic decisions. However, the German trial only showed advantages in terms of disease-free survival, not OS [16]. It remains unclear whether the NAR score offers better predictive accuracy for OS compared to other prognostic factors.

While immunotherapy has shown significant benefits in various cancer types, such as melanoma and lung cancer [18], these improvements were not seen in most rectal cancer patients [19]. Unlike other cancers, radiotherapy can exert an impact on the tumor microenvironment by eliciting systemic immune-mediated antitumor effects [20]. Until now, several clinical trials are underway to evaluate the role of neoadjuvant immunotherapy combined with radiotherapy in rectal cancer. Preliminary studies had shown that the combination of immunology and radiotherapy had led to promising results. It is believed that resistance to immunotherapy in microsatellite stable rectal cancer may be overcome by the potential immune-stimulating effect of radiation. Although most of the reported studies are small samples from phase 1–2 clinical trials, complete response (CR) rates have shown a good trend toward improvement [21]. A Chinese clinical trial of short-course radiotherapy (SCRT) combined with immunotherapy had shown a high pCR of 48% (13/27), which exceeded the results of the total neoadjuvant therapy model [22]. Studies to date have shown that lymphocyte levels are related to neoadjuvant response for cancer patients, including rectal, oesophageal, and breast cancer [23–25]. Emerging evidence suggests that high tumor-infiltrating lymphocytes (TIL) levels, particularly CD8+ T-cells, is related to improved prognosis for neoadjuvant rectal cancer patients [26]. A previous study has reported a correlation between higher NAR scores and various clinic-pathologic features, such as old age, positive cN status, and lower tumor differentiation [16]. However, a model incorporating immune cell infiltration characteristics to predict long-term prognosis for rectal cancer has not yet been developed.

Therefore, we aimed to modify the NAR scoring model by incorporating immune cell infiltration characteristics and applying it to LARC patients who received nRT, to establish a more efficient approach for promptly identifying the success or failure of experimental interventions and, thereby, improve clinical decision support.

2. Materials and Methods

2.1. Eligibility Criteria

In this retrospective study, we extracted and recorded detailed information from hospital records of LARC who underwent nRT with subsequent TME in our institution between February 2012 and September 2015. Criteria comprised histologically confirmed adenocarcinoma and staging II (T3-4N0) or III (TanyN+) rectal cancer based on the American Joint Committee on Cancer staging system, 8th Edition. Pelvic MRIs were conducted before and after nRT in all eligible patients. Patients diagnosed with metastatic disease or a Karnofsky performance score below 70 were excluded. All clinicopathological features and laboratory results were recorded. Finally, seventy-six cases were included in the study. Our study had the approval of the Ethics Committee of Fujian Cancer Hospital (NO. KT2022-120-01).

Moreover, the Ethics Committee waived individual consent as the analysis involved retrospective examination of patient information, without utilizing any individual patient identifiable information.

2.2. Treatment and Follow-Up

Patients underwent either short-course radiotherapy (SCRT; 25 Gy in 5 fractions) or long-course chemoradiotherapy (LCRT; 50 Gy in 25 fractions) as neoadjuvant therapy. Concurrent chemotherapy during LCRT consisted of capecitabine (825 mg/m²) administered two times a day, 5 days a week. The treatment options are determined by the clinician. Typically, patients with high tumor burden are selected for long course radiotherapy. The primary cancer was included in the clinical target volume (CTV), as well as the anorectal, mesorectal, pre-sacral, and internal iliac lymph nodes. Intensity-modulated radiation therapy (IMRT) was used to deliver the RT regimens. All plans were created for a Varian Truebeam accelerator using 6 MV photon beam. After nRT, TME was performed in all cases, either one week after SCRT or 6 to 8 weeks after LCRT, by expert surgeons.

For the first 2 years, all patients were followed every 3 months, and then every 6 months for 3 years, and then on an annual basis thereafter.

2.3. NAR Scores

The NAR score compromised of weighted cT to take into account the downstaging of tumors, as well as ypT and ypN categories, which are affected by neoadjuvant therapy [15]. The NAR score equation is as follows: NAR = [5ypN – 3(cT – ypT) + 12]² / 9.61, where cT in {1,2,3,4}, ypT in {0,1,2,3,4}, and pN in {0,1,2} [14]. Subsequently, the NAR scores encompass 24 different scores ranging from 0–100. The pCR is equal to 0 points, while 100 points represent progress.

2.4. TRG

Two pathologists independently evaluated H&E staining of surgical specimens after nRT. A five-grade Mandalay system (Fibrosis/Tumor Relationship Tumor Relationship) was used for TRG—TRG 1: complete regression, no viable cancer cells; TRG 2: rare residual cancer cells scattered through the fibrosis; TRG 3: increased number of residual cancer cells, fibrosis predominates; TRG 4: residual cancer outgrowing fibrosis; and TRG 5: no regression [27]. The patients were divided into two groups: those with complete regression (TRG 1) and those with incomplete regression (TRG 2–5).

2.5. Clinicopathological Variable Stratification

Receiver operating characteristic (ROC) analysis from the Cutoff Finder (<http://molpath.charite.de/cutoff>, accessed on 6 April 2023) [28], were employed to classify PD-1, CD3+ T-cell, and CD8+ T-cell as dichotomous variables, with optimal cutoff values of 5.5%, 12.5%, and 9.0%, respectively.

The variables in the cohort were categorized as follows: age at diagnosis (<60, ≥60), gender (male or female), body mass index (BMI, <18.5 kg/m², 18.5–24.9 kg/m², ≥25 kg/m²), distance to anal margin (<5 cm, ≥5 cm), lymphovascular invasion (yes or no), neural invasion (yes or no), tumor nodules (yes or no), mucinous adenocarcinoma (yes or no), pathologic differentiation (poorly, moderately), cT stage (cT2, T3, T4), cN stage (cN0, N1, N2), ypT stage (ypT0, ypT2, ypT3, ypT4), ypN stage (ypN0, ypN1, ypN2), PD-1 (<5.5%, ≥5.5%), CD3+ T-cell (<12.5%, ≥12.5%), CD8+ T-cell (<9.0%, ≥9.0%), pCR (yes, no), nRT (short course, long course), neoadjuvant chemotherapy (yes or no), adjuvant radiotherapy (yes or no), and adjuvant chemotherapy (yes or no).

2.6. Statistical Analyses

OS refers to the time from diagnosis to the time of death. Cox regression models were used for analysis of survival and identification of variables with prognostic significance. Statistical significance was defined as a *p* value less than 0.05. Subsequently, based on the multivariate analysis, a nomogram was constructed showing OS rates at 3, 5, and 10 years. The predictive ability was evaluated using Harrell's C-index. Receiver operating characteristic (ROC) analysis was performed using the Delong test. The efficiency of the nomogram was compared with other factors.

SPSS version 22.0 (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses. The R software v.4.2.2 (<https://www.r-project.org/>, accessed on 6 April 2023) installation package "rms" was used to construct the nomogram. *p* < 0.05 was considered statistically significant.

3. Results

3.1. Patient and Tumor Characteristics

The study enrolled 76 patients (49 males, 27 females). Table 1 presents the characteristics of the patients. The median follow-up was 86 months (range, 3–125). The NAR score was in the range of 0 to 65 (IQR, 8.43–30.07; median 20.4). Median age was 53 years (range, 19–74 years). Of the cases, $\text{BMI} < 18.5 \text{ kg/m}^2$ occurred in 7 cases (9.2%), BMI between 18.5–24.9 kg/m^2 occurred in 53 cases (69.7%), and $\text{BMI} \geq 25 \text{ kg/m}^2$ occurred in 16 cases (21.1%). Approximately 64.5% of patients had low-lying tumors (within 5 cm of the anal verge). Lymphovascular invasion was present in 15 patients (19.7%), neural invasion in 14 patients (18.4%), and tumor nodules in 16 patients (21.1%). Mucinous adenocarcinoma was observed in 8 patients (10.5%), while 16 patients (21.1%) had poorly differentiated tumors. The distribution of cT stages was as follows: cT2 (4 cases), cT3 (41 cases), and cT4 (31 cases). Lymph-node-positive status was diagnosed in 48 cases (63.2%). After nRT, 55 patients (72.3%) had pathological (yp) T3-4 tumors and 33 patients (43.4%) had ypN0 status. A pCR was achieved in 7 patients (9.2%). Regarding nRT, 36 patients (47.4%) underwent short-course radiotherapy (SCRT), while 40 patients (52.6%) received long-course radiotherapy (LCRT). Neoadjuvant chemotherapy was administered to 63 cases (82.9%). Adjuvant radiotherapy was given to 6 patients (7.9%) and adjuvant chemotherapy to 52 patients (68.4%).

Table 1. Characteristics of rectal cancer with neoadjuvant radiotherapy.

| Characteristics | Descriptive Analysis | |
|------------------------------|----------------------|------|
| | n | % |
| NAR score | MEAN 20.4 ± 16.3 | |
| Age | | |
| <60 | 56 | 73.7 |
| ≥60 | 20 | 26.3 |
| Gender | | |
| Male | 49 | 64.5 |
| Female | 27 | 35.5 |
| BMI (kg/m^2) | | |
| <18.5 | 7 | 9.2 |
| 18.5–24.9 | 53 | 69.7 |
| ≥25 | 16 | 21.1 |
| Distance to anal margin (cm) | | |
| <5 | 49 | 64.5 |
| ≥5 | 27 | 35.5 |
| Lymphovascular invasion | | |
| Yes | 15 | 19.7 |
| No | 61 | 80.3 |

Table 1. *Cont.*

| Characteristics | Descriptive Analysis | |
|----------------------------|----------------------|------|
| | n | % |
| Neural invasion | | |
| Yes | 14 | 18.4 |
| No | 62 | 81.6 |
| Tumor nodules | | |
| Yes | 16 | 21.1 |
| No | 60 | 78.9 |
| Mucinous adenocarcinoma | | |
| Yes | 8 | 10.5 |
| No | 68 | 89.5 |
| Pathologic differentiation | | |
| Poorly | 16 | 21.1 |
| Moderately | 60 | 78.9 |
| cT stage | | |
| 2 | 4 | 5.3 |
| 3 | 41 | 53.9 |
| 4 | 31 | 40.8 |
| cN stage | | |
| 0 | 28 | 36.8 |
| 1 | 38 | 50.0 |
| 2 | 10 | 13.2 |
| ypT stage | | |
| 0 | 4 | 5.3 |
| 2 | 17 | 22.4 |
| 3 | 47 | 61.8 |
| 4 | 8 | 10.5 |
| ypN stage | | |
| 0 | 33 | 43.4 |
| 1 | 27 | 35.5 |
| 2 | 16 | 21.1 |
| PD-1 | | |
| <5.5% | 61 | 80.2 |
| ≥5.5% | 15 | 19.8 |
| CD3+ T-cell | | |
| <12.5% | 14 | 18.4 |
| ≥12.5% | 62 | 81.6 |
| CD8+ T-cell | | |
| <9.0% | 41 | 53.9 |
| ≥9.0% | 35 | 46.1 |
| pCR | | |
| Yes | 7 | 9.2 |
| No | 69 | 90.8 |
| Neoadjuvant radiotherapy | | |
| Short course | 36 | 47.4 |
| Long course | 40 | 52.6 |
| Neoadjuvant chemotherapy | | |
| Yes | 63 | 82.9 |
| No | 13 | 17.1 |
| Adjuvant radiotherapy | | |
| Yes | 6 | 7.9 |
| No | 70 | 92.1 |
| Adjuvant chemotherapy | | |
| Yes | 52 | 68.4 |
| No | 24 | 31.6 |
| Death | | |
| Yes | 34 | 44.7 |
| No | 42 | 55.3 |

Abbreviations: NAR, Neoadjuvant rectal score; BMI, Body mass index; PD-1, Programmed cell death 1; pCR, Pathological complete regression.

3.2. Cutoff Values for Immune Cell Infiltration Characteristics

Using the Cutoff Finder software via ROC curves, we determined the optimal cutoff value for PD-1 as 5.5% in our cohort (range, 0–20%; median 1%; mean 3.1%). Among the cases, 61 (80.2%) had a low PD-1 expression (<5.5%) and 15 (19.8%) had a high PD-1 expression ($\geq 5.5\%$). The software also identified an optimal cutoff value of 12.5% for CD3+ T-cells (range, 0%–90%; median 30%; mean 33%). Fourteen cases (18.4%) were classified as having a low CD3+ T-cell count (<12.5%), while 62 cases (81.6%) had a high CD3+ T-cell count ($\geq 12.5\%$). Additionally, the optimal cutoff value for CD8+ T-cell was 9.0% (range, 0–80%; median 10%; mean 19%). Among the cases, 41 cases (53.9%) had a low CD8+ T-cell count (<9.0%), while 35 cases (46.1%) had a high CD8+ T-cell count ($\geq 9.0\%$). The results are illustrated in Figure 1 and Table 1.

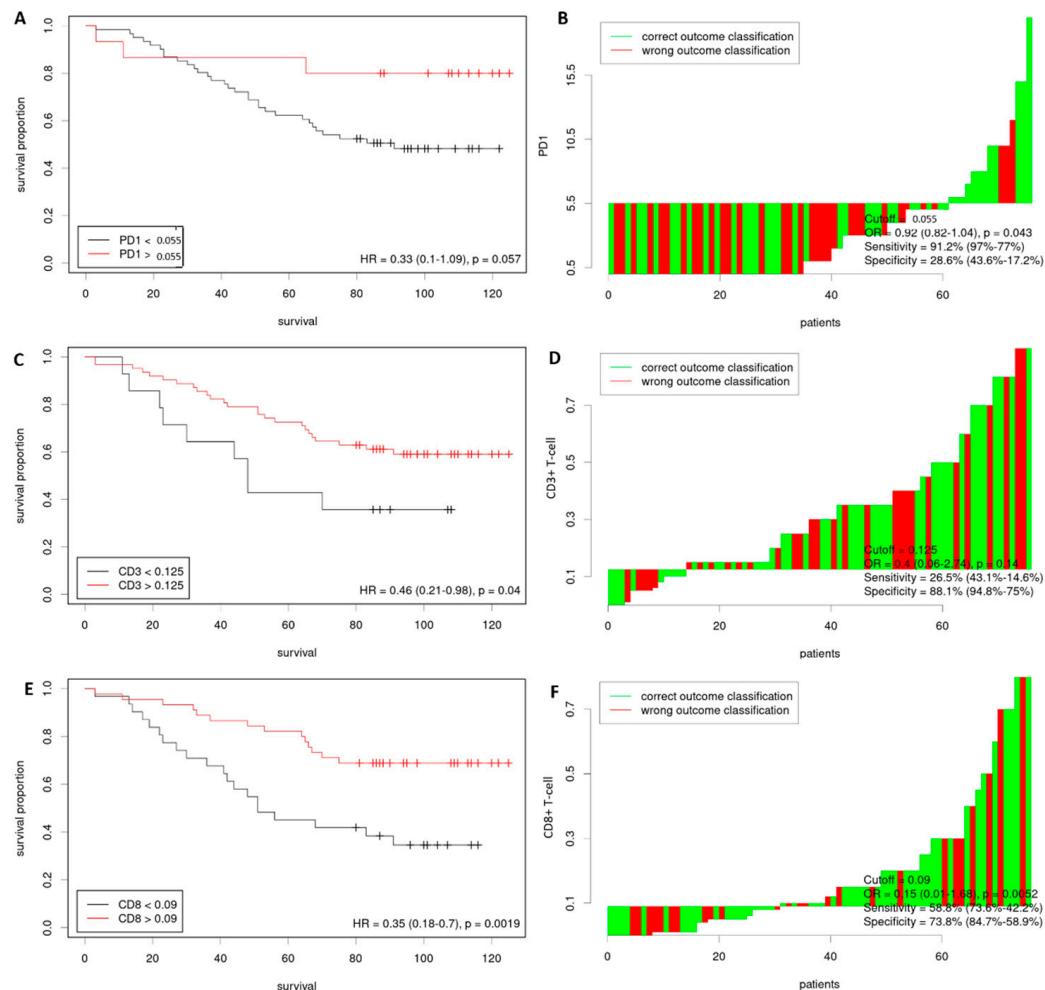


Figure 1. Distribution-based cutoff optimization in LARC patients. Kaplan–Meier curves comparing low vs. high PD-1 (A), CD3+ T-cell (C), and CD8+ T-cell (E). Waterfall plots illustrate the optimal dichotomization of PD1 (B), CD3+ T cell (D), and CD8+ T cell (F) levels. The optimal cutoff was determined based on the occurrence of death events. Abbreviations: LARC, locally advanced rectal cancer; PD1, programmed cell death 1.

3.3. Independent Prognostic Factors for OS

The results of the univariate and multivariate analyses are presented in Tables 2 and 3. In the univariate analysis, lymphovascular invasion (HR = 5.148, 95% CI = 2.516–10.533, $p = 0.000$), neural invasion (HR = 2.232, 95% CI = 1.064–4.681, $p = 0.034$), pathologic differentiation (HR = 2.273, 95% CI = 1.086–4.761, $p = 0.029$), ypN stage (HR = 2.460, 95% CI = 1.587–3.814, $p = 0.000$), CD3+ T-cell (HR = 0.459, 95% CI = 0.213–0.987, $p = 0.046$), CD8+ T-cell (HR = 0.354, 95% CI = 0.178–0.703, $p = 0.003$), and NAR score (HR = 1.036, 95% CI = 1.017–1.056, $p = 0.000$) were significantly associated with OS. Additionally, to avoid statistical bias resulting from multicollinearity, NAR, and pathologic stage were not examined within the same model [11]. Multivariate analysis indicated that CD8+ T-cell (HR 0.433, 95% CI 0.198–0.948, $p = 0.036$), lymphovascular invasion (HR 3.375, 95% CI 1.254–9.087, $p = 0.016$), and NAR score (HR 1.028, 95% CI 1.005–1.051, $p = 0.019$) were independent predictors for OS.

Table 2. Variables of LARC Patients Associated with OS According to the Cox Proportional Hazards Regression Model.

| Characteristics | Univariable Analysis | | |
|------------------------------|----------------------|--------------|-------|
| | HR | 95% CI | p |
| Age | 1.774 | 0.878–3.587 | 0.110 |
| Gender | 0.629 | 0.301–1.317 | 0.219 |
| BMI | 1.306 | 0.685–2.491 | 0.417 |
| Distance to anal margin (cm) | 1.248 | 0.625–2.494 | 0.530 |
| Lymphovascular invasion | 5.148 | 2.516–10.533 | 0.000 |
| Neural invasion | 2.232 | 1.064–4.681 | 0.034 |
| Tumor nodules | 1.844 | 0.879–3.868 | 0.106 |
| Mucinous adenocarcinoma | 1.990 | 0.769–5.154 | 0.156 |
| Pathologic differentiation | 2.273 | 1.086–4.761 | 0.029 |
| cT stage | 0.592 | 0.332–1.056 | 0.076 |
| cN stage | 1.220 | 0.742–2.007 | 0.432 |
| ypT stage | 1.136 | 0.762–1.694 | 0.531 |
| ypN stage | 2.460 | 1.587–3.814 | 0.000 |
| PD-1 | 0.334 | 0.102–1.095 | 0.070 |
| CD3+ T-cell | 0.459 | 0.213–0.987 | 0.046 |
| CD8+ T-cell | 0.354 | 0.178–0.703 | 0.003 |
| NAR score | 1.036 | 1.017–1.056 | 0.000 |
| pCR | 0.250 | 0.034–1.832 | 0.173 |
| Neoadjuvant Radiotherapy | 0.582 | 0.295–1.147 | 0.118 |
| Neoadjuvant chemotherapy | 0.667 | 0.290–1.532 | 0.340 |
| Adjuvant radiotherapy | 1.080 | 0.330–3.535 | 0.898 |
| Adjuvant chemotherapy | 0.893 | 0.435–1.833 | 0.759 |

Abbreviations: HR, Hazard ratio; CI, Confidence interval; OS, Overall survival; NAR, Neoadjuvant rectal score; pCR, Pathological complete regression.

Table 3. Risk Factors for Survival According to Multivariate Logistic Regression Analyses.

| Variable | β Coefficient | HR | 95% CI | p |
|----------------------------|---------------------|-------|-------------|-------|
| Lymphovascular invasion | 1.217 | 3.375 | 1.254–9.087 | 0.016 |
| Neural invasion | −0.393 | 0.675 | 0.218–2.089 | 0.496 |
| Pathologic differentiation | 0.251 | 1.286 | 0.578–2.861 | 0.538 |
| CD3+ T-cell | −0.298 | 0.742 | 0.279–1.976 | 0.551 |
| CD8+ T-cell | −0.836 | 0.433 | 0.198–0.948 | 0.036 |
| NAR score | 0.027 | 1.028 | 1.005–1.051 | 0.019 |

Abbreviations: HR, Hazard ratio; CI, Confidence interval; NAR, Neoadjuvant rectal score.

3.4. The Nomogram for OS

Subsequently, all independent prognostic factors were incorporated into the nomogram model based on the multivariate analysis (Figure 2). The nomogram scoring was significantly related to TRG grading through Spearman relative analysis ($p = 0.013$). The C-index for OS was 0.739 (95% CI 0.657–0.821), which was higher than that for the AJCC stage (0.587, 95% CI 0.537–0.636), and pCR (0.539, 95% CI 0.495–0.583). Additionally, the C-index for nomogram was superior to that for CD8+ T-cell (0.630, 95% CI 0.549–0.711), lymphovascular invasion (0.606, 95% CI 0.523–0.688) and NAR score (0.696, 95% CI 0.609–0.782). Moreover, the area under the curves (AUCs) were plotted to compare the predictive ability of the nomogram and the other grading systems (Figure 3). The AUCs for OS demonstrated that this nomogram was significantly more predictive than the AJCC stage and pCR at 3-years, 5-years, and 10-years, respectively (3-years OS: nomogram AUC = 0.834, AJCC stage AUC = 0.607, pCR AUC = 0.557, $p = 0.0289$, 0.0039; 5-years OS: nomogram AUC = 0.825, AJCC stage AUC = 0.627, pCR AUC = 0.539, $p = 0.0001$, 8.673836×10^{-10} ; 10-years OS: nomogram AUC = 0.937, AJCC stage AUC = 0.470, pCR AUC = 0.485, $p = 5.367856 \times 10^{-58}$, 2.100348×10^{-79}). Over time, the predictions of long-term survival using this model not only remained consistent but also improved in accuracy.

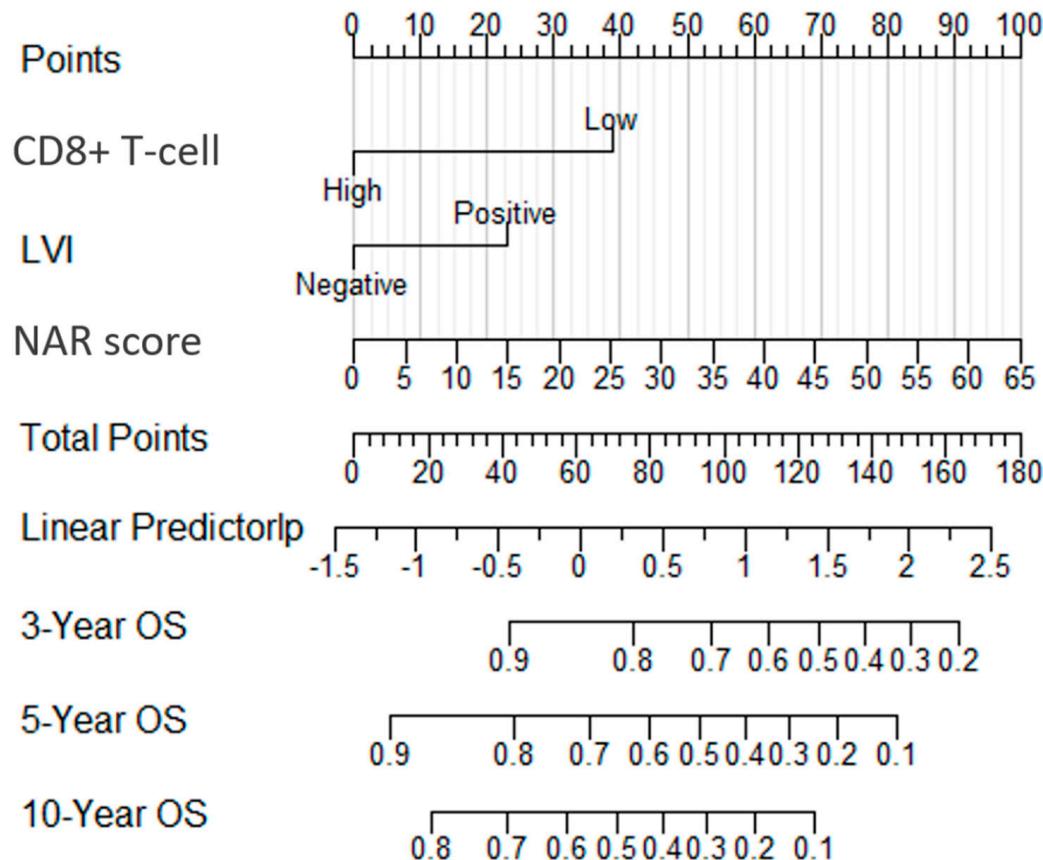


Figure 2. Nomogram incorporating CD8+ T-cell, LVI, and NAR score for predicting risk of death in patients with LARC. The nomogram provides a tool for calculating the probability of death risk by considering the combined effect of these variables. Abbreviations: LVI, Lymphovascular invasion; NAR score, Neoadjuvant rectal score; LARC, Locally advanced rectal cancer; OS, Overall survival.

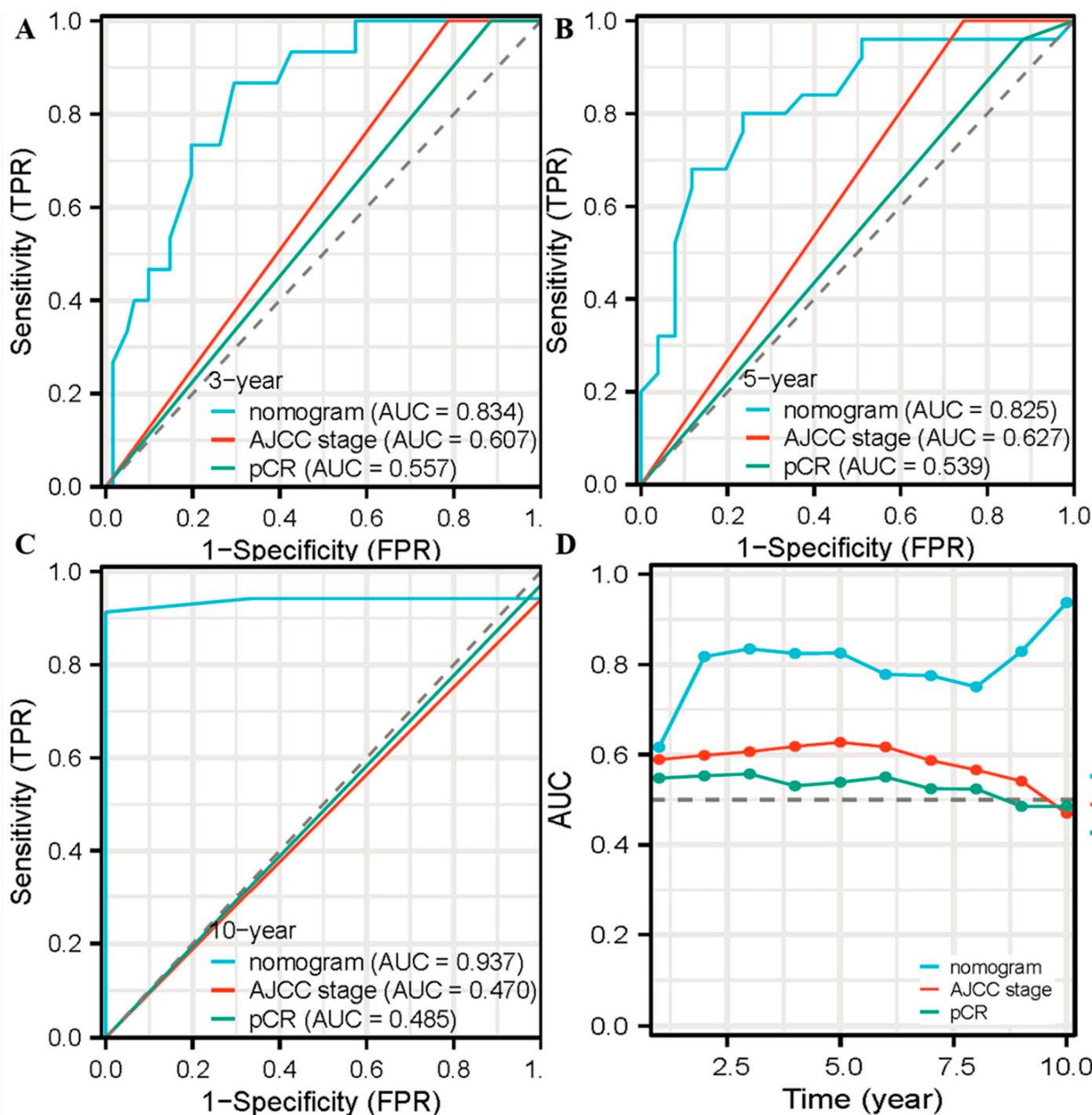


Figure 3. AUC values for the three models predicting OS rates at 3 years (A), 5 years (B), and 10 years (C). (D) Time-dependent ROC. Abbreviations: AUC, Area under the curve; OS, Overall survival; ROC, Receiver operating characteristic; AJCC, American Joint Committee on Cancer; pCR, Pathological complete regression.

4. Discussion

Neoadjuvant chemoradiotherapy followed by TME have been the standard strategies for LARC patients. The response to nRT varies from pCR to minimal effect. Incorporating a novel surrogate short-term end-point is important for assessing the long-term prognosis of LARC treated with nRT. This is the first nomogram combining NAR score and immune cell infiltration characteristics to predict long-term survival to our knowledge. The modified

NAR scoring model demonstrates improved accuracy in predicting long-term prognosis compared to the AJCC stage and pCR, making it a more reliable tool over time.

In our cohort, the NAR score (HR 1.028, 95% CI 1.005–1.051, $p = 0.019$) was significantly associated with OS. In our scoring model, CD8+ T-cell (HR 0.433, 95% CI 0.198–0.948, $p = 0.036$), and lymphovascular invasion (HR 3.375, 95% CI 1.254–9.087, $p = 0.016$) were newly added as independent prognostic factors to the modified NAR scoring model. Furthermore, we observed that the new modified-NAR scoring model exhibited a high C-index of 0.739, which was superior to any individual independent factor. Moreover, the predictive value of the nomogram was better than that of the AJCC stage and pCR at 3 years, 5 years, and 10 years ($p < 0.05$). Over time, the predictions of this model for long-term survival not only remained consistent but also improved in accuracy.

DFS and OS are commonly used endpoints in rectal cancer clinical trials, but they require long periods of observation, which slows the pace of research. Previous research has presented varying data regarding the predictive ability of the NAR score in rectal cancer. The score utilizes factors commonly used in clinical work for rectal cancer and requires no additional trial infrastructure, cost, time, or effort. According to the German phase III trial, the NAR score continued to predict DFS independently (low versus high NAR: HR = 4.670, 95% CI 3.106–7.020, $p < 0.001$; low versus intermediate NAR: HR 1.971, 95% CI 1.303–2.98, $p = 0.001$) [16]. However, the baseline staging in that study did not mandate the use of magnetic resonance imaging (MRI), which may have affected the accuracy of the clinical stage. In our cohort, cT and cN stages were assessed via MRI by two radiologists independently to decrease the uncertainty associated with ultrasound staging. Nonconformities were reviewed by a third expert. In a large hospital-based dataset, an unfavorable NAR score (≥ 14.98) was associated with poor OS ($p = 0.04$) and tumors with perineural invasion had worse NAR scores ($p = 0.01$) [29]. Similarly, among the low, intermediate, and high NAR groups, a significant difference in 5-year OS was observed (93% vs. 88% vs. 75%; $p < 0.001$), and NAR stratification was confirmed to be a significant prognostic factor for 5-year OS (intermediate vs. low, HR = 1.82, 95% CI 1.57–2.10, $p < 0.001$; high vs. low, HR = 3.44, 95% CI 2.94–4.03, $p < 0.001$) [30]. In the NRG-GI002 trial, the mean NAR score was 11.53 for the pembrolizumab arm (95% CI 8.54–14.51) vs. 14.08 for the control arm (95% CI 10.74–17.43) ($p = 0.26$), which was the primary endpoint, although analyses of longer-term results are underway [17]. Therefore, after the long follow-up time, we tried to examine the long-term results here. Furthermore, previous research has shown that the NAR score has a better prognostic value for OS in rectal cancer clinical trials compared to pCR [15]. A nomogram combining the NAR score, TRG, and distance to the anal verge was developed to predict OS and DFS (AUC: 1-year = 0.742, 3-year = 0.749, 5-year = 0.713) [31]. Consistent with previous studies, our study found a significant association between the NAR score and OS (HR 1.028, 95% CI 1.005–1.051, $p = 0.019$). By combining variables related to treatment effects, the NAR score can be used as a surrogate endpoint for short-term efficacy assessments.

Currently, the immune contexture, representing the pre-existing immune parameter, was reported to be associated with cancer prognosis. And, the immunoscore, which combined multiple immune cells, might help to predict the cancer survival [32]. The immunoscore was found to be a predictive factor for neoadjuvant response and long-term prognosis in LARC patients, which was expected to play a role in selecting patients for watch-and-wait strategies [33]. Studies have highlighted the important prognostic value of the immune system in rectal cancer, with tumor-infiltrating lymphocytes (TILs) identified as prognostic factors for favorable oncological outcomes [19,34]. A high density of CD8+ cell (OR 2.69, 95% CI 1.45–4.98, $p = 0.002$) in biopsy samples has been linked with good CRT response, which was considered a good biomarker for predicting outcome after neoadjuvant therapy for rectal cancer [35]. Chemotherapy and radiotherapy have been shown to activate the immune system and synergize with immunotherapy [36]. High density of CD8+ T-cells before CRT has been associated with improved DFS ($p = 0.0331$) [37]. Additionally, the density of CD8+ T-cells increases after chemoradiotherapy (CRT) ($p < 0.001$), and low

expression of CD8+ T-cells before and after CRT has been linked to unfavorable DFS ($p = 0.01$) [38]. Moreover, neoadjuvant CRT leads to increased recruitment of CD8+ T-cells within the tumor microenvironment, and high CD8+ T-cell density has been significantly associated with improved DFS after neoadjuvant CRT ($p = 0.039$) [39]. Similarly, in our cohort, the CD8+ T-cell was found to be associated with OS for LARC after nRT (HR 0.433, 95% CI 0.198–0.948, $p = 0.036$).

The incorporation of the NAR score and CD8+ T-cell density into the nomogram resulted in improved predictive accuracy, as evidenced by a high C-index for OS of 0.739. The modified NAR scoring model showed more predictive power than the NAR score (C-index = 0.696) and CD8+ T-cell (C-index = 0.630). Additionally, this was superior to the AJCC stage (C-index = 0.587) and pCR (C-index = 0.539). Furthermore, the modified nomogram exhibited significantly stronger AUC values than the AJCC stage and pCR for 3-year, 5-year, and 10-year OS ($p < 0.05$). Consequently, our nomogram, with its reliance on long-term follow-up, presents a simple and accurate tool for predicting prognosis in LARC patients after nRT. This represents a significant advantage over the AJCC stage and pCR.

Nonetheless, the study has several limitations. Firstly, the results of this single-center retrospective study require further validation. Secondly, although we analyzed immune cell infiltration characteristics such as PD1, CD3+ T-cell, and CD8+ T-cells, some important prognosis-related data were not recorded, such as more immune cells, microsatellite status (MSS), RAS, and BRAF genes. Moreover, the novel modified scoring model would benefit from further validation in prospective studies conducted across multicenter with a large sample size.

5. Conclusions

In summary, we successfully developed a modified-NAR scoring model that incorporates immune infiltration characteristics to better predict long-term survival in rectal cancer after nRT. The validation of this model at long-term follow-up demonstrates its accuracy and reliability, which improves over time.

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Institutional Review Board Statement: This is a retrospective study and which was conducted in accordance with the 1964 Declaration of Helsinki and International Conference on Harmonization of Good Clinical Practice Tripartite guidelines. This clinical study was approved by the Ethics Committee of Fujian Cancer Hospital Fuzhou, China (No. K2022-120-01, approved on 29 December 2022). All patients gave informed consent to participate in this study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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