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**Name of Journal:** *World Journal of Gastrointestinal Oncology*

**Manuscript NO:** 78939

**Manuscript Type:** ORIGINAL ARTICLE

*Retrospective Study*

**Predictive value of indirect bilirubin before neoadjuvant chemoradiotherapy in evaluating prognosis of local advanced rectal cancer patients**

Li SF *et al.* Predictive of pre-IBIL in LARC prognosis

## **Abstract**

### **BACKGROUND**

Many biomarkers have predictive value for overall survival (OS) and disease-free survival (DFS) in tumor patients. However, the role of indirect bilirubin (IBIL) in local advanced rectal cancer (LARC) patients treated with neoadjuvant chemoradiotherapy (nCRT) has not been studied.

### **AIM**

To explore the predictive value of IBIL before nCRT (pre-IBIL) for the OS and DFS of LARC patients treated with nCRT.

### **METHODS**

A total of 324 LARC patients undergoing nCRT with total mesorectal excision (TME) were enrolled. Preoperative clinical features and postoperative pathological characteristics were collected. Cox regression analysis was performed, and a Cox-based nomogram was developed to predict OS and DFS. We also assessed the predictive performance of the nomogram with calibration plots and receiver operating characteristic (ROC) curves.

### **RESULTS**

Among 324 patients, the median pre-IBIL was 6.2  $\mu\text{mol/L}$  (interquartile range: 4.6  $\mu\text{mol/L}$ -8.4  $\mu\text{mol/L}$ ). In the Cox multivariate regression analysis, we found that pre-IBIL, smoking history, tumor regression grade (TRG), vascular invasion, and carbohydrate antigen 19-9 before nCRT (pre-CA19-9) were predictors of OS. Additionally, pre-IBIL, body mass index (BMI), nCRT with surgery interval, TRG, and vascular invasion were predictors of DFS. Predictive nomograms were developed to predict 5-year OS and 5-year DFS with area under the ROC curve values of 0.7518 and 0.7355, respectively. Good statistical performance on internal validation was shown by calibration plots and ROC curves.

## CONCLUSION

This study demonstrated that pre-IBIL was an independent prognostic factor for OS and DFS in LARC patients treated with nCRT followed by TME. Nomograms incorporating pre-IBIL, BMI, smoking history, nCRT with surgery interval, TRG, vascular invasion, and pre-CA19-9 could be helpful to predict OS and DFS.

**Key Words:** Indirect bilirubin; Local advanced rectal cancer; Neoadjuvant chemoradiotherapy; Prognostic factor; Overall survival; Disease-free survival

Li SF, Wei R, Yu GH, Jiang Z. Predictive value of indirect bilirubin before neoadjuvant chemoradiotherapy in evaluating prognosis of local advanced rectal cancer patients. *World J Gastrointest Oncol* 2022; In press

**Core Tip:** Our study demonstrated that indirect bilirubin measurement before neoadjuvant chemoradiotherapy (nCRT) (pre-IBIL) is an independent and significant risk factor for survival in local advanced rectal cancer patients treated with nCRT followed by total mesorectal excision. In addition, nomograms based on pre-IBIL can predict 5-year overall survival and 5-year disease-free survival with good agreement.

## INTRODUCTION

According to the National Comprehensive Cancer Network guidelines, local advanced rectal cancer (LARC) patients should be treated with neoadjuvant chemoradiotherapy (nCRT) combined with surgery plus adjuvant chemotherapy<sup>[1]</sup>. The nCRT can reduce the tumor burden, and local recurrence rate, as well as improve the R0 resection rate and anus preservation rate of LARC<sup>[2]</sup>. However, approximately 20%-46% of LARC patients with available postoperative pathological data had tumor cells that did not regress significantly or did not regress at all. These patients did not respond significantly to treatment and had poor prognoses<sup>[3-5]</sup>. Moreover, there are few studies

on the changes in biological characteristics of tumors after nCRT treatment, so the current prognosis of this group of patients is still evaluated by TNM staging, which may not be accurate<sup>[6-8]</sup>. Combined with the results of existing studies, most of the studies evaluated the prognosis of this group of patients based on the pathological or anatomical characteristics of the tumor while ignoring some biological characteristics of the patients themselves, such as blood biochemistry status and underlying diseases<sup>[9-11]</sup>.

Indirect bilirubin (IBIL) is bilirubin that is not bound to glucuronic acid. Elevated serum IBIL is mainly associated with various hemolytic diseases. After the destruction of a large number of red blood cells, a large amount of hemoglobin is converted into IBIL, which exceeds the processing capacity of the liver to convert all of it into direct bilirubin, resulting in elevated IBIL in the blood. Its concentration reflects the conversion function of hepatocytes and the catabolic state of red blood cells. In recent years, an increasing number of studies have found a relationship between IBIL and tumor prognosis. Some studies have shown that IBIL is associated with prognosis in cancer patients, such as ovarian cancer, lung cancer, nasopharyngeal cancer, and oral squamous cell carcinoma<sup>[12-15]</sup>. However, the predictive values of the IBIL before nCRT (pre-IBIL) for prognostic outcomes in LARC patients treated with nCRT are unknown. In this retrospective study, we aimed to perform statistical analysis of the clinicopathological data of a large number of LARC patients who underwent nCRT was performed to investigate the predictive value of pre-IBIL in the prognosis and to create a nomogram that could predict the prognosis of patients.

## **MATERIALS AND METHODS**

### ***Patient population***

We retrospectively identified 324 rectal cancer patients who received nCRT between November 1, 2012 and October 30, 2018 in the National Cancer Center/Cancer Hospital of the Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, P.R. China). The inclusion criteria were as follows: (1) Completion of long course nCRT followed by total mesorectal excision (TME); (2) Pathologically confirmed

rectal adenocarcinoma by colonoscopic biopsy; and (3) Imaging suggested cTNM stage II-III without distant metastasis at initial diagnosis. The exclusion criteria were as follows: (1) Absence of some clinical features before nCRT treatment; (2) After nCRT, distant metastasis was found by imaging examinations; and (3) Synchronous tumors.

All the available detailed clinical characteristics and pathological parameters were enrolled, including pre-IBIL, age, sex, body mass index (BMI), smoking history, drinking history, chronic disease history, cTNM stage, nCRT with surgery interval, inferior margin, tumor regression grade (TRG), vascular invasion, neural invasion, radiotherapy type before surgery, chemotherapy type before surgery, adjuvant chemotherapy, carcinoembryonic antigen before nCRT (pre-CEA), and carbohydrate antigen 19-9 before nCRT (pre-CA19-9), in which TRG uses Dworak stage.

### ***Treatment***

All patients were treated with a 45-60 Gy dose of long-course nCRT. Three schemes of concurrent radiotherapy are volumetric modulated arc therapy (VMAT), intensity modulated radiation therapy (IMRT), and 3-dimensional conformal radiation therapy (3D-CRT). The concurrent chemotherapy schemes were capecitabine, capecitabine + platinum, capecitabine + bevacizumab, capecitabine + oxaliplatin and raltitrexed. All patients were treated with nCRT for at least 4 wk before undergoing TME.

### ***Follow-up***

All patients received postoperative reviews in the hospital every 3 mo for 2 years after surgery and every 6 mo for 3-5 years after surgery. The postoperative examinations included a physical examination, peripheral blood tumor markers, chest computed tomography (CT), abdominal CT, pelvic CT or magnetic resonance imaging, and a whole-body positron emission tomography-CT if necessary. Additionally, we followed up with patients at regular intervals until August 31, 2021. Overall survival (OS) was defined as the time from the date of diagnosis to the date of death due to any cause or

the last follow-up. Disease-free survival (DFS) was defined as the time since radical surgery to disease recurrence, metastasis, the last follow-up, or patient death.

### ***Statistical analysis***

Fisher's exact test and  $\chi^2$  tests were employed to compare categorical data. Cox regression analysis was performed to evaluate the hazard ratio (HR) and 95% confident interval (95%CI) of both OS and DFS. Parameters that were statistically significant in the univariate analysis were subsequently included in the multivariate analysis. Nomograms were constructed based on statistically significant factors identified by the multivariate analyses from the Cox regression model to predict 5-year OS and DFS. We assessed the predictive performance of the nomogram with calibration plots and receiver operating characteristic (ROC) curves. The Kaplan-Meier survival method was used to analyze OS and DFS, and survival differences were calculated by the log-rank test. All statistical analyses were performed using R version 4.1.0. Two-tailed *P* values less than 0.05 were considered statistically significant.

## **RESULTS**

### ***Patient characteristics***

A total of 324 patients, 98 (30.25%) females and 226 (69.75%) males met the inclusion criteria for this study, and the median pre-IBIL (interquartile range) was 6.2 (4.6-8.4)  $\mu\text{mol/L}$ . Then, 6.2  $\mu\text{mol/L}$  was used as cut-off value in grouping patients. Moreover, 48.46% (157/324) of patients had a smoking history, 41.67% (135/324) of patients had a drinking history and 37.04% (120/324) of patients had a chronic disease history. For radiotherapy type before surgery, 286 (88.27%) patients received the VMAT regimen, 30 (9.26%) patients received the IMRT regimen, and 8 (2.47%) patients received the 3D-CRT regimen. For chemotherapy type before surgery, 289 (89.20%) patients received the capecitabine regimen, and 18 (5.56%) patients received the capecitabine plus platinum regimen. The demographics and clinicopathological characteristics are presented in Table 1.

### *Cox regression analysis of prognostic factors for OS*

All variables identified in Table 1 were selected for univariable Cox regression analysis to estimate OS in LARC patients treated with nCRT and TME. In the univariable analysis, there were many variables statistically significant for OS in these patients, including pre-IBIL, smoking history, TRG, vascular invasion, neural invasion, and pre-CA19-9. These statistically significant parameters in the univariate analysis were included in the multivariate model. In the multivariate analysis, we found that pre-IBIL (pre-IBIL > 6.2  $\mu\text{mol/L}$ , adjusted HR = 1.77, 95%CI: 1.04-3.01,  $P = 0.035$ ), smoking history (yes, adjusted HR = 0.58, 95%CI: 0.34-1.00,  $P = 0.048$ ), TRG (TRG = 2, adjusted HR = 0.37, 95%CI: 0.18-0.75,  $P = 0.006$ ; TRG=3, adjusted HR = 0.27, 95%CI: 0.12-0.64,  $P = 0.003$ ; TRG = 4, adjusted HR = 0.18, 95%CI: 0.06-0.53,  $P = 0.002$ ), vascular invasion (positive, adjusted HR = 2.93, 95%CI: 1.39-6.21,  $P = 0.005$ ), and pre-CA19-9 (pre-CA19-9 > 27 U/mL, adjusted HR = 2.05, 95%CI: 1.16-3.62,  $P = 0.014$ ) were predictors of OS (Table 2).

### *Cox regression analysis of prognostic factors for DFS*

All variables identified in Table 1 were selected for univariable Cox regression analysis to estimate DFS in LARC patients treated with nCRT and TME. Univariable analysis revealed that factors including pre-IBIL, BMI, nCRT with surgery interval, TRG, vascular invasion, and neural invasion were independently associated with DFS in these patients. All statistically significant predictors in the univariate analysis were included in the multivariate model. The multivariate analysis revealed that pre-IBIL (pre-IBIL > 6.2  $\mu\text{mol/L}$ , adjusted HR = 1.86, 95%CI: 1.22-2.84,  $P = 0.004$ ), BMI (BMI  $\geq$  28.0, adjusted HR = 0.19, 95%CI: 0.05-0.66,  $P = 0.009$ ), nCRT with surgery interval (nCRT with surgery interval = 61-90 d, adjusted HR = 0.63, 95%CI: 0.40-0.99,  $P = 0.044$ ), TRG (TRG = 2, adjusted HR = 0.43, 95%CI: 0.24-0.78,  $P = 0.005$ ; TRG = 3, adjusted HR = 0.30, 95%CI: 0.15-0.62,  $P = 0.001$ ; TRG = 4, adjusted HR = 0.31, 95%CI: 0.14-0.70,  $P =$



0.005), and vascular invasion (positive, adjusted HR = 2.24, 95% CI: 1.13-4.44,  $P = 0.021$ ) were predictors of DFS (Table 3).

### *Construction of nomograms to predict OS and DFS*

We established nomograms for the prediction of the 5-year OS and DFS using the independent variables (Figure 1). Pre-IBIL, smoking history, TRG, vascular invasion, and pre-CA19-9 were statistically significant predictors of OS on Cox multivariate analysis. Pre-IBIL, BMI, nCRT with surgery interval, TRG, and vascular invasion were statistically significant predictors of DFS on Cox multivariate analysis. Therefore, these variables were subsequently included in the nomogram. A weighted total score is calculated from these factors, which is applied to predict the OS and DFS for the LARC patients who received nCRT. We found that patients with a lower pre-IBIL had better OS and DFS.

### *Evaluation of the nomogram*

The nomogram for predicting OS and DFS in LARC patients treated with nCRT was developed based on the multivariate model in the cohort. The area under the ROC curve (AUC) was analyzed for the cohort, and the AUC values for the 5-year OS and DFS nomograms were 0.7518 and 0.7355, respectively (Figure 2). The model demonstrated good statistical performance for predicting OS and DFS, and calibration curves for the probability of 5-year OS and 5-year DFS indicated satisfactory consistency between actual observation and nomogram prediction (Figure 3).

### *Kaplan-Meier survival curves*

In Kaplan-Meier survival analysis, we found that pre-IBIL was related to OS (Figure 4A) and DFS (Figure 4B) in LARC patients treated with nCRT. For OS, the median was 5.28 years. The patients in the pre-IBIL  $\leq 6.2$   $\mu\text{mol/L}$  group had a significantly longer OS than patients in the pre-IBIL  $> 6.2$   $\mu\text{mol/L}$  group ( $P = 0.042$ ). The 5-year survival rate of the patients in the pre-IBIL  $\leq 6.2$   $\mu\text{mol/L}$  group was 87.27%, higher than that of

the patients in the pre-IBIL > 6.2  $\mu\text{mol/L}$  group, 78.48%. For DFS, the median DFS was 4.71 years. Additionally, the patients in the pre-IBIL  $\leq$  6.2  $\mu\text{mol/L}$  group also had a significantly longer DFS than patients in the pre-IBIL > 6.2  $\mu\text{mol/L}$  group ( $P = 0.012$ ). The 5-year recurrence rates of patients in the pre-IBIL  $\leq$  6.2  $\mu\text{mol/L}$  and pre-IBIL > 6.2  $\mu\text{mol/L}$  groups were 76.90% and 64.39%, respectively.

## **DISCUSSION**

The nCRT combined with TME is the standard treatment for patients with LARC<sup>[16]</sup>. Although this treatment regimen has been shown to improve the local control rate in some LARC patients, there are still considerable differences in prognoses among patients<sup>[17]</sup>. **Tumor markers are substances that are synthesized or released by tumor cells themselves or produced by the body in response to tumor cells during the process of tumor development. When these substances reach a certain level, they can indicate the existence of a specific tumor, and the changes in the levels of these substances can be used to monitor the recurrence and progression of the tumor<sup>[18]</sup>. At present, common tumor markers are not routinely tested in health checkups, which often leads to the missed diagnosis of some patients with early malignant tumors, thus indicating that it is important to identify new tumor markers during routine checkups.**

Previous studies have focused attention on identifying pretreatment blood-based biomarkers and traditional tumor biomarkers, including CEA, CA19-9 and the neutrophil-to-lymphocyte ratio. These biomarkers have been proven to have prognostic effects in predicting the response and survival in LARC<sup>[19]</sup>. In recent years, liquid biopsy has received increasing attention in cancer research, and biomarkers based on liquid biopsy have also shown favorable significance in treatment and prognosis prediction<sup>[20,21]</sup>. For example, a prospective study including 119 Chinese LARC patients identified circulating tumor DNA as a good predictor for risk stratification and for guiding the treatment strategy<sup>[22]</sup>. However, these new biomarkers are still under investigation, and their clinical application may need to be further studied to obtain a consensus.

Biochemical tests for blood samples are routinely performed for each hospitalized patient, and bilirubin is one of the most important markers of the biochemical test. Bilirubin is a major toxic metabolite of iron porphyrin compounds in the body, which<sup>4</sup> can cause irreversible damage to the nervous system; however, it has antioxidant properties and can inhibit the oxidation of linoleic acid and phospholipids. Recent studies have shown that bilirubin, as an endogenous antioxidant, can moderately increase the ability of cancer patients to scavenge oxidative free radicals in the body and affect the prognoses of cancer patients. Therefore, bilirubin levels are of great clinical significance as a novel tumor biomarker<sup>[23-27]</sup>. One study demonstrated that the direct bilirubin-to-IBIL ratio was an independent predictor of survival in resectable colorectal cancer patients<sup>[28]</sup>. Nevertheless, few studies have been conducted to explore the prognostic effect of bilirubin in LARC patients.

In this study, we collected clinical and pathological data of 324 LARC patients treated with nCRT combined with TME and followed up with each of these patients. We performed all factor Cox regression analyses in Table 1, and the characteristics with statistically significant results from the<sup>10</sup> univariate analysis were included in the multivariate analysis. The multivariate analysis results showed that pre-IBIL, smoking history, TRG, vascular invasion and pre-CA19-9 were risk factors affecting OS. The multivariate analysis results showed that pre-IBIL, BMI, nCRT with surgery interval, TRG and vascular invasion were risk factors affecting DFS. In addition, we constructed nomograms to evaluate prognoses based on the above indicators.

Our research had the following advantages. First, we found a correlation of pre-IBIL with the prognosis of LARC patients after receiving nCRT, and the prognoses of those patients with pre-IBIL > 6.2  $\mu\text{mol/L}$  were poorer, which indicated that pre-IBIL is a risk factor affecting the prognoses of patients. Second, we performed a Cox regression analysis of pre-IBIL with common characteristics and found that pre-IBIL was associated with the OS and DFS of LARC patients receiving nCRT. We also constructed nomograms that could predict OS and DFS. Third, we collected clinical characteristics, pathological characteristics, treatment information, hematological characteristics and

sociological characteristics of patients and considered more comprehensive factors.<sup>8</sup> However, our study had some limitations. First, this was a single-center study, the sample size was relatively small and the results need to be further validated by expanding the sample size. Second, in our study, with the limitations of the retrospective nature, we found an association between a blood sample and oncological outcomes in patients with LARC, and future prospective studies should be conducted to further explore the associations between pre-IBIL and the prognoses of LARC patients.

## <sup>1</sup> **CONCLUSION**

In conclusion, this study demonstrated that pre-IBIL was an independent prognostic factor for OS and DFS in LARC patients treated with nCRT followed by TME. Nomograms incorporating pre-IBIL, BMI, smoking history, nCRT with surgery interval, TRG, vascular invasion, and pre-CA19-9 could be helpful to predict OS and DFS. These findings provide a new direction for future clinical precision treatment and scientific research.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Neoadjuvant chemoradiotherapy (nCRT) has been regarded as the standard treatment for local advanced rectal cancer (LARC). Indirect bilirubin (IBIL) has shown significance in the prognosis of various cancer types, including ovarian cancer and lung cancer. However, the predictive values of IBIL in the prognoses of LARC patients treated with nCRT remain unknown.

### ***Research motivation***

The present study attempted to identify the prognostic value of IBIL before nCRT (pre-IBIL) in LARC patients and to construct a nomogram based on pre-IBIL to predict the survival of the patients.

### *Research objectives*

This study aimed to identify the prognostic value of pre-IBIL in LARC patients and to construct a nomogram to predict their 5-year overall survival (OS) and 5-year disease-free survival (DFS).

### *Research methods*

A total of 324 LARC patients undergoing nCRT with total mesorectal excision (TME) were enrolled. Preoperative clinical features and postoperative pathological characteristics were collected. A Cox regression analysis was performed, and a Cox-based nomogram was developed to predict OS and DFS. We also assessed the predictive performance of the nomogram with receiver operating characteristic (ROC) and curves calibration plots.

### *Research results*

In the Cox multivariate regression analysis, we found that pre-IBIL, smoking history, tumor regression grade (TRG), vascular invasion and carbohydrate antigen 19-9 before nCRT were predictors of OS. Furthermore, pre-IBIL, body mass index, nCRT with surgery interval, TRG and vascular invasion were predictors of DFS. Predictive nomograms were developed to predict 5-year OS and 5-year DFS with areas under the ROC curve of 0.7518 and 0.7355, respectively. Good statistical performance on internal validation was shown via the calibration plots and ROC curves.

### *Research conclusions*

Pre-IBIL was an independent prognostic factor for OS and DFS in LARC patients treated with nCRT followed by TME. Nomograms based on pre-IBIL could be helpful for predicting survival in LARC patients.

### *Research perspectives*

Although our single-center study identified the prognostic value of pre-IBIL in LARC patients, a future prospective study with larger samples should be conducted to further explore the association between pre-IBIL and the prognosis of LARC patients.



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