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Clinical Value of CTR, CEA, Histological Type, Ki-67 and EGFR in Predicting pN in Clinical Stage IA Lung Adenocarcinoma and Nomogram Construction

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Abstract

To investigate the clinical value of CTR, CEA, histological type, Ki-67 and EGFR in detecting pathological lymph node metastasis (pN) in clinical stage IA (cIA) lung adenocarcinoma and to construct a pN Nomogram model**.** A total of 374 cIA lung adenocarcinoma patients who had undergone thoracoscopic radical resection with Systematic mediastinal lymph node dissection (SMLD) in the Department of Thoracic Surgery of the Affiliated Hospital of Qingdao University between January 2018 to January 2020 were retrospectively reviewed. The patients were divided into pN(+) and pN(-) groups. Univariate and multivariate Logistic regression analyses were used to analyze the independent risk factors of pN in lung cancer patients. The ROC curve was used to compare the accuracy of CTR, CEA and Ki-67 in predicting pN. R software was used to construct a Nomogram prediction model based on multivariate Logistic regression analysis of the pN risk. The C-index was calculated, and a calibration curve was drawn to judge the calibration degree of the model. The preoperative and intraoperative examinations showed that CTR (OR 570.406, P＜0.001), CEA (OR 1.239, P＜0.001) and micropapillary adenocarcinoma (OR 86.712, P＜0.001) were independent risk factors of pN. Immunohistochemical analysis and gene detection showed that Ki-67 index (OR 4.832, P＜0.001) and EGFR mutations, such as exon 19 (OR 10.319, P＜0.001), exon 21 (OR 7.163, P＜0.001) and exon 19+20 mutations (OR 570.406, P＜0.001), were significant factors in predicting pN. CTR, CEA, histological type, Ki-67 index, and EGFR mutations are the predictive factors of pN in cT1a-3aN0M0 lung adenocarcinoma patients. SMLD is recommended to improve patients' postoperative survival rate when preoperative CTR≥0.775, CEA＞2.52μg/L or intraoperative rapid freezing pathology shows micropapillary components.

Introduction

More and more early non-small cell lung cancers (NSCLCs) have been identified, especially in cIA, due to the introduction of chest high-resolution computed tomography (HRCT) and health awareness improvement. Anatomical pulmonary lobectomy with SMLD is the standard procedure for NSCLC. However, pathological metastatic positive lymph node affects prognosis [1] and can significantly reduce the survival rate of lung cancer patients [2, 3]. The lymph node metastasis rate of NSCLC (diameter≤2cm) is between 15%-20% [4, 5]. Some scholars have shown that SMLD has greater surgical trauma, a higher incidence rate of postoperative complications, longer postoperative catheterization and hospitalization. Besides, extensive lymphadenectomy can decrease local immune function, leading to local recurrence and distant metastasis, indicating that SMLD is not necessary for all early NSCLCs [6, 7]. Metastase-negative lymph node dissection is inefficient and can prolong operation or increase the perioperative complication risks [7, 8]. Therefore, the clinicopathological data of 374 cT1a-3aN0M0 primary lung adenocarcinoma patients were retrospectively analyzed, the risk factors of pathologically metastatic positive lymph nodes were explored, and an individualized Nomogram model was constructed to predict the pN occurrence rate. This study provides a standardized theoretical basis for the formulation of intraoperative lymph node dissection and postoperative adjuvant therapy for cIA lung adenocarcinoma patients.

Methods

Patients

A retrospective case-control study was conducted. The clinicopathological data of 374 cT1a-3aN0M0 lung adenocarcinoma patients who underwent anatomical pulmonary lobectomy with SMLD in the Department of Thoracic Surgery of the Affiliated Hospital of Qingdao University between January 2018 to January 2020 were collected (8th edition of TNM). A preoperative evaluation was conducted on all patients via thyroid ultrasound,

chest CT, abdominal CT, craniocerebral CT and bone scanning. Chest CT showing lymph node with short axial ≥1cm indicated metastatic positive. Inclusion criteria were: age 70 years old, single nodule ≤3cm in diameter, hilar and mediastinal lymph nodes with long axis diameter ≤1cm in HRCT, non-distant metastasis, and pathologically confirmed primary lung adenocarcinoma. Exclusion criteria included preoperative immunotherapy, targeted therapy or chemoradiotherapy, a history of other malignant tumor and incomplete clinicopathological data.

Histopathological assessment

The resected tumor tissue and lymph nodes were stained using hematoxylin and eosin. Immunohistochemical and genetic tests were performed, and two pathologists reviewed the biopsy specimens. Histopathological information, Ki-67 index and EGFR mutation status were noted. Besides, the resected lung tissues were classified into acinar-dominant adenocarcinoma (APA), papillary type-dominant adenocarcinoma (PPA), micropapillary type-dominant adenocarcinoma (MPA), solid type-dominant adenocarcinoma (SPA) and anchorage type-dominant adenocarcinoma (LPA), according to the International Association for the Study of Lung Cancer (IASLC)/The American Thoracic Society (ATS)/the European Respiratory Society (ERS).

Statistical analyses

IBM SPSS 23.0 software was used for data analysis. The measurement data (skewed distribution) was expressed as a median. Mann-Whitney U test was used to compare the two groups. Chi-square test or Fisher's exact test were used to compare the enumeration data. P 0.05 was considered statistically significant. Univariate analysis was used to determine pN risk factors. Multivariate Logistic regression analysis of statistically significant factors was conducted to identify the independent risk factors for lymph node metastasis. The SPSS software was used to draw the receiver operating characteristic (ROC), including CTR, CEA, Ki-67, CTR+CEA combined prediction probability and CTR+CEA+Ki-67 combined prediction probability. The area under the curve (AUC) and the best cut-off value of ROC were then calculated. The MedCalc software was used to construct the ROC curves of CTR, CEA and Ki-67 to compare and evaluate their differences in predicting pN. The R software was used to construct an individualized Nomogram model of CTR, CEA, and histological types based on multivariate Logistic regression analysis of preoperative risk assessment of pN. The C-index was then calculated, and the calibration degree of the model was plotted.

Results

Clinicopathological characteristics of patients

A total of 374 patients met the inclusion criteria, 171(45.7%) male and 203(54.3%) female. The average age was 60 years old. About 76.7% were non-smokers, 294 had no alcohol history, 222 had lung cancer on the right side (59.4%), and 152(40.6%) on the left side, with the right upper lobe being the most common site (32.6%). Postoperative pathological diagnosis revealed that 145 patients had lymph node metastasis with a metastasis rate of 38.8%. Most of the 145 pN(+) patients had no history of smoking or drinking. Preoperative imaging examination showed that the median tumor size and CTR were 2.50 cm (0.70-3.00cm) and 0.90, respectively. Moreover, the histological types were micropapillary type (35.2%), followed by acinus type and papillary type, and EGFR exon 21 mutation was the most common, according for 48.2% (Table 1).

Univariate and multivariate Logistic regression analysis of pN

Univariate analysis indicated that tumor size (P□0.001), CTR (P□0.001), tumor markers, such as CEA (P□0.001) and CYFRA21-1 (P=0.016), histological type (P□0.001), Ki-67 index≥12.5% (P□0.001) and EGFR mutation (P□0.001) could predict pN. These statistically significant indicators were incorporated into multivariate Logistic regression analysis to reveal five crucial pN predictors: CTR (OR 570.406, 95% CI 30.582-10638.897, P□0.001), preoperative serum CEA level (OR 1.239, 95% CI 1.111-1.382, p□0.001), micropapillary type (OR 86.712, 95% CI 8.579-876.408, P□0.001), Ki-67 index (OR 4.832, 95% CI 2.160-10.806, P□0.001) and EGFR mutations, such as exon 19 mutation (OR 10.319, 95% CI 3.368-31.611, P□0.001), exon 21 mutation (OR 7.163, 95% CI 2.867-17.897, P□0.001) and exon 19+20 co-mutation (OR 12.202, 95% CI 1.501-99.162, P=0.019). Tumor size was not an independent risk factor in multivariate analysis, possibly because it was affected by CTR. Furthermore, acinar adenocarcinoma could be a protective pN factor (Table 2).

Clinical value of continuous variables predicting pN

ROC curve was drawn based on the distribution of continuous variables that could predict pN. The AUC of CTR, CEA, Ki-67 index, CTR+CEA combined prediction probability and CTR+CEA+Ki-67 combined prediction probability were 0.850 (95% CI 0.812-0.888), 0.823 (95% CI 0.778-0.867), 0.791 (95% CI 0.745-0.838), 0.893 (95% CI 0.862-0.924) and 0.908 (95% CI 0.879-0.937). CTR+CEA+Ki-67 combined prediction probability had the highest AUC, sensitivity, and specificity with great clinical value in predicting pN. The CTR+CEA combined prediction probability of AUC, sensitivity and specificity were 0.893, 0.841 and 0.786, respectively, and could also preoperatively predict the pN incidence. Besides, the incidence of pN significantly increased when CTR≥0.775, CEA□2.520 or Ki-67 index ≥12.5% (Fig.1 and Table 3).

ROC curve differences between CTR, CEA and Ki-67 in predicting pN

MedCalc software was used to compare the ROC curve differences between CTR, CEA, and Ki-67 in predicting pN. The AUC of CEA was not significantly different from CTR and Ki-67, indicating that CEA was as accurate as CTR and Ki-67 in predicting pN. However, CTR was statistically different in predicting lymph node metastasis (P=0.03) and had higher accuracy and greater clinical value in predicting pN than Ki-67 (Fig.2 and Table 4).

Construction and verification of Nomogram prediction model

R software was used to establish a Nomogram model to predict pN risk based on the multivariate Logistic regression analysis results of CTR, CEA and histological types (Fig.3). The model indicated that the total score of the three risk factors decreased, and the risk of pN increased, with increasing CTR or CEA. The C-index of the predicted model was 0.922 (95% CI 0.896-0.948). The calibration curve revealed that the model had a good calibration degree. Furthermore, the average absolute error between the actual occurrence risk and the predicted occurrence risk was 0.01 (Fig.4).

Discussion

The pathological status of lymph nodes is essential for the formulation of postoperative treatment strategy and prognosis of NSCLC [9]. Some scholars have shown that clinicopathological factors of patients can predict the lymph node metastasis status in early NSCLC [10]. Similarly, in this study, CTR, preoperative serum CEA level, histological type, Ki-67 index and EGFR mutations were key in predicting pathological lymph node metastasis.

Tumor size is a risk factor for pN in clinically node-negative NSCLC [11-13]. However, the predictive effect of tumor size on lymph node metastasis in NSCLC≤3cm is unknown. Yu et al. [14] indicated that 53 patients (13.4%) had

lymph node metastasis in early NSCLC (diameter, 1-2cm), while no metastasis in tumor≤1cm. Zhang [15] reported that lymph node metastasis rates of NSCLC of diameter≤1cm and 1-2cm were 3.8% and 7.4%, respectively. However, in this study, multivariate Logistic regression analysis showed that tumor size was not an independent pN risk factor (P=0.089). Similarly, Haruki [16] showed that larger tumors (diameter≥2.0cm, ≤5.0cm) was not associated with mediastinal lymph node metastasis (P=0.158). Lung adenocarcinoma growth is characterized by diameter and solid components increasement. GGO-dominant tumor patients mainly show solid components increasement, indicating that tumor size generally does not affect pN. The high CTR ratio-tumors are mainly characterized by diameter increasement, and tumor size is related to lymph node metastasis. Therefore, another possible reason is that the included patients could be GGO-dominant.

Preoperative serum CEA level might be an indicator of lymph node dissection. Haruki showed that the elevated CEA level in primary lung adenocarcinoma in the lower lobe of both lungs was significantly correlated with upper mediastinal lymph node metastasis (P<0.001) [16]. Koike also indicated that CEA was a risk factor for lymph node metastasis in cIA NSCLC [17]. In this study, the AUC, sensitivity, specificity and best cut-off value of CEA were 0.823, 0.786, 0.760 and 2.52 μg/L, respectively, indicating good pN predicting ability. The lymph node metastasis risk increases with increasing CEA levels when CEA ≥2.52 μg/L preoperatively. Moreover, some scholars have suggested that CEA≤5 μg/L belongs to normal range and is not associated with pN. The single-center and small sample study could cause the error. Therefore, the relationship between CEA an pN should be further studied. Several studies have shown that preoperative serum CEA level is a risk factor for N2 lymph node metastasis [18-21] but is not associated with N1 lymph node metastasis [18].

Zhang et al. [18] indicated that tumor consolidation is a risk factor for N1 and N2 lymph node metastasis. Meanwhile, Haruki et al. [16] showed that the mediastinal lymph node metastasis rate of adenocarcinoma is 9.9% (74/744). Besides, hilar and mediastinal lymph node metastasis does not occur in GGO-dominated lung cancer patients. In this study, the ROC curve was used to determine CTR predictive efficacy of lymph node metastasis. CTR was shown to be a preoperative pN predictor, with no statistical difference in predictive power between with CEA (P=0.336). The cut-off value of CTR was calculated (0.775). The lymph node metastasis rate increased when the cut-off value≥0.775. In consequence, SMLD should be intraoperatively adopted when HRCT indicates CTR≥0.775 before operation.

In addition, adenocarcinoma in situ and microinvasive adenocarcinoma rarely have lymph node metastasis [22]. However, invasive adenocarcinoma, especially MPA, is associated with a higher lymph node metastasis rate and poor prognosis [11, 23]. The analysis of 297 cN0-1 lung adenocarcinoma patients showed that MPA was related with pathological N2 lymph node metastasis, and the rate increased with increasing micropapillary components [24]. In our study, we also found that AIS and MIA were not associated with lymph node metastasis. Besides, the proportion of PPA and MPA was higher in pN(+) than in pN(-). Multivariate analysis indicated that MPA was an independent risk factor for lymph node metastasis. Therefore, lymph node dissection or sampling is not required for AIS and MIA, while SMLD is recommended for invasive adenocarcinoma, especially MPA.

Xue et al. [25] reported lymph node metastasis rates of 11.9% (28/235), 23.5% (215/915), 36.4%(536/1473) at Ki-67 of≤5%, 5%-25% and ≥25%, respectively, and the differences were statistically significant (P<0.001). Similarly, in this study, the Ki-67 index was a pN risk factor with a cut-off value of 12.5%. Ki-67 was less effective in predicting pN risk than CTR (P=0.03) but similar to CEA (P=0.299). Besides, EGFR mutations were most common in exon 19 (40.4%) and exon 21 (48.2%). Han and colleagues [26] indicated that EGFR mutations mainly occur in exon 19 (E746-A750 and I744- K745 ins KIPVAI) and exon 21 (L858R), with a coincidence rate of 95% in primary NSCLC and

N2 metastatic lymph nodes. Guerrera et al. [27] also showed that EGFR mutations are significantly associated with skipping lymph node metastasis (P□0.001), but not overall survival of lung adenocarcinoma. These studies have contributed to the formulation of a postoperative treatment plan and prognosis evaluation.

Nomogram is a simple visualization model used to predict diseases based on multiple factors. The predictive model for postoperative anastomotic leakage in colorectal cancer has been widely reported [28, 29]. However, it has rarely been reported on lymph node metastasis risk in lung cancer. In our study, a personalized prediction model was constructed using available clinicopathological data to analyze and evaluate the pN risk. Preoperative and intraoperative risk indicators can help to select the appropriate method of lymph node dissection. Postoperative indicators can also guide clinicians to formulate reasonable postoperative adjuvant treatment plans.

However, this study has some limitations: First, it is a retrospective case-control study, and there could be selection bias. Secondly, this is a small sample and single-center study so that a large-sample randomized multicenter prospective study is necessary to provide strong evidence. Finally, this research did not consider postoperative survival and recurrence, which may lead to ignore some lymph node dissection indicators.

Conclusions

In conclusions, 374 cIA lung adenocarcinoma patients were retrospectively analyzed. The pathological lymph node metastasis risk significantly increased when CTR≥0.775, CEA□2.52μg/L, rapid freezing pathology examination showed micropapillary type, Ki-67 index≥12.5%, or EGFR exon 19, 21, 19+20 co-mutation occurred. The established Nomogram model can be used for individualized prediction of preoperative lymph node metastasis. Furthermore, the combined Ki-67 index and EGFR mutation status analysis can guide surgical planning and postoperative adjuvant therapy development. However, further studies are needed to explore other potential risk factors for predicting pathological lymph node metastasis.

Declarations

Author contributions Tengfei Yi designed the experiment and drafted the manuscript. Shuo Li and Kun Qin executed the statistical analyses. Guisong Song, Shengteng Shao and Dongdong Dai interpreted the experiments. Yuhong Liu revised the manuscript.

Date availability The data used and/or analyzed during the current study are available by contacting the corresponding author with reasonable request.

Compliance with ethical standards

Conflict of interest The author declare no conflicts of interests.

Consent for publication The suthors declare that they consent to the manuscript publication.

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Tables

Table1 Clinicopathological factors associated with pathological lymph node metastasis in cT1a-3aN0M0 lung adenocarcinoma patients (n=374)

Variables	N	pN-(n=229) n(%)	pN+(n=145) n(%)	χ2	p-Value
Age	-	60	61	-	0.111
Gender				0.332	0.565
Female	203	127（55.5）	76（52.4）		
Male	171	102（44.5）	69（47.6）		
Smoking history				3.335	0.068
Never	287	183（79.9）	104（71.7）		
Former or current	87	46（20.1）	41（28.3）		
Alcohol history					
Never	294	186（81.2）	108（74.5）	2.399	0.121
Former or current	80	43（18.8）	37（25.5）		
Complication	207	125（54.6）	82（56.6）	0.139	0.709
With	167	104（45.4）	63（43.4）		
Without					
Tumor location	84	52（22.7）	32（22.1）	2.742	0.602
Left upper	68	36（15.7）	32（22.1）		
Left lower	122	79（34.5）	43（29.6）		
Right upper	20	13（5.7）	7（4.8）		
Right middle	80	49（21.4）	31（21.4）		
Right lower					
Tumor size	-	1.50	2.50	-	<0.001
CTR	-	0.64	0.90	-	<0.001
Tumor markers	-	1.67	4.41	-	<0.001
CEA	-	2.16	2.61	-	0.016
CYFRA21-1					
Histology subtype	5	5（2.2）	0（0.0）	132.459	<0.001
AIS	28	28（12.2）	0（0.0）		
MIA	162	120（52.4）	42（29.0）		
APA	83	48（21.0）	35（24.1）		
PPA	52	1（0.4）	51（35.2）		
MPA	25	20（8.7）	5（3.4）		
LPA	19	7（3.1）	12（8.3）		

SPA					
Ki-67				79.405	<0.001
≤12.5%	247	191（83.4）	56（38.6）		
≥12.5%	127	38（16.6）	89（61.4）		
KRAS(mutation)（n=56）				0.957	0.328
Yes	56	31（13.5）	25（17.2）		
No	318	198（86.5）	120（82.8）		
EGFR（mutation）（n=215）				52.382	<0.001
Exon18	7	6（5.9）	1（0.9）		
Exon19	81	35（34.7）	46（40.4）		
Exon20	14	9（8.9）	5（4.4）		
Exon21	103	48（47.5）	55（48.2）		
Exon19+ Exon20	10	3（3.0）	7（6.1）		

Table2 Multivariate Logistic regression analysis of pathological lymph node metastasis in cIA lung adenocarcinoma

Relevant factors	B	SE	Wald	P	OR	95% CI	
Tumor size	0.586	0.344	2.898	0.089	1.797	0.915	3.529
CTR	6.346	1.493	18.072	0.000	570.406	30.582	10638.897
CEA	0.214	0.056	14.834	0.000	1.239	1.111	1.382
CYFRA21-1	0.055	0.089	0.390	0.532	1.057	0.888	1.258
Subtype			15.445	0.017			
Subtype（1）	0.045	0.422	0.012	0.914	1.046	0.458	2.392
Subtype（2）	4.463	1.180	14.297	0.000	86.712	8.579	876.408
Subtype（3）	-0.372	0.802	0.215	0.643	0.689	0.143	3.320
Subtype（4）	0.628	0.726	0.749	0.387	1.875	0.452	7.779
Subtype（5）	-18.075	6403.887	0.000	0.998	0.000	0.000	—
Subtype（6）	-12.490	17895.890	0.000	0.999	0.000	0.000	—
Ki-67(1)	1.575	0.411	14.709	0.000	4.832	2.160	10.806
EGFR			27.822	0.000			
EGFR（1）	2.334	0.571	16.697	0.000	10.319	3.368	31.611
EGFR（2）	0.192	1.065	0.033	0.857	1.212	0.150	9.768
EGFR（3）	1.969	0.467	17.760	0.000	7.163	2.867	17.897
EGFR（4）	2.502	1.069	5.476	0.019	12.202	1.501	99.162
EGFR（5）	-1.871	1.715	1.191	0.275	0.154	0.005	4.434

Table3 ROC analysis in pathological lymph node metastasis prediction

	cut-off	AUC	SE	Sig	95%CI	Youden index	Sensitivity	Specificity
CTR	0.775	0.850	0.019	0.001	0.812-0.888	0.563	0.821	0.742
CEA	2.520	0.823	0.023	0.001	0.778-0.867	0.546	0.786	0.760
Ki-67	12.500	0.791	0.024	0.001	0.745-0.838	0.448	0.614	0.834
CTR+CEA	0.359	0.893	0.016	0.001	0.862-0.924	0.627	0.841	0.786
CTR+CEA+Ki67	0.415	0.908	0.015	0.001	0.879-0.937	0.662	0.828	0.834

Table4 Pairwise comparison of ROC curves in CTR, CEA and Ki-67

	Difference of AUC	SE	95%CI	Z statistic	P
CTR~Ki-67	0.059	0.027	0.006-0.111	2.171	0.030
CTR~CEA	0.027	0.028	-0.028-0.083	0.963	0.336
Ki-67~CEA	0.031	0.030	-0.028-0.091	1.039	0.299

Figures

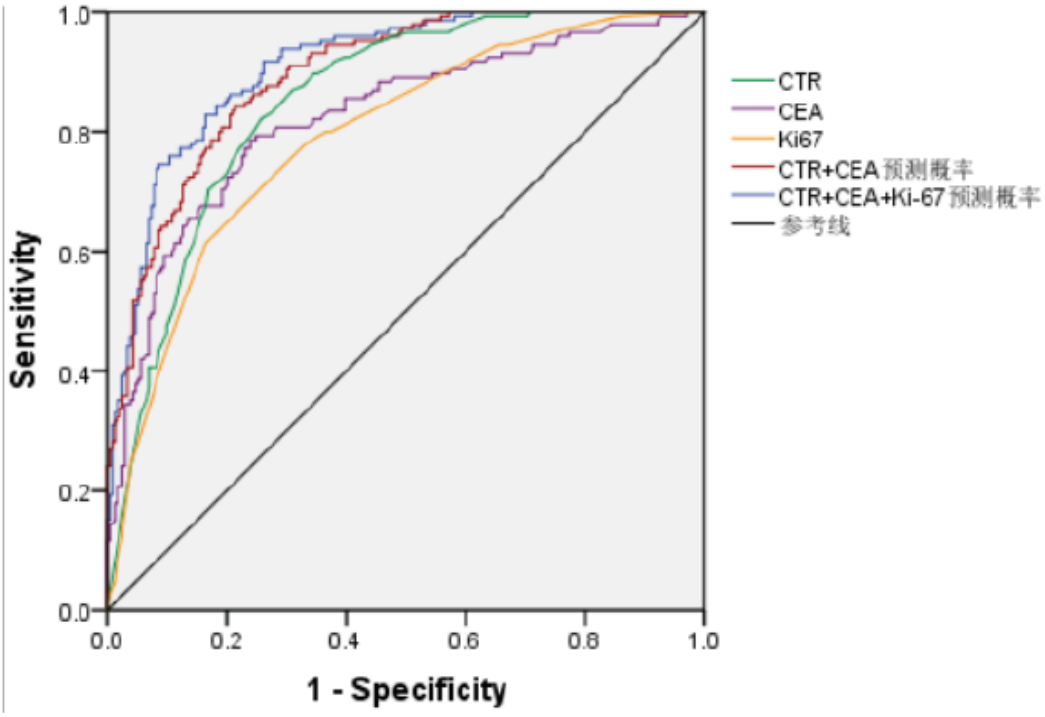

Figure 1

ROC curve of risk factors predicting pathological lymph node metastasis

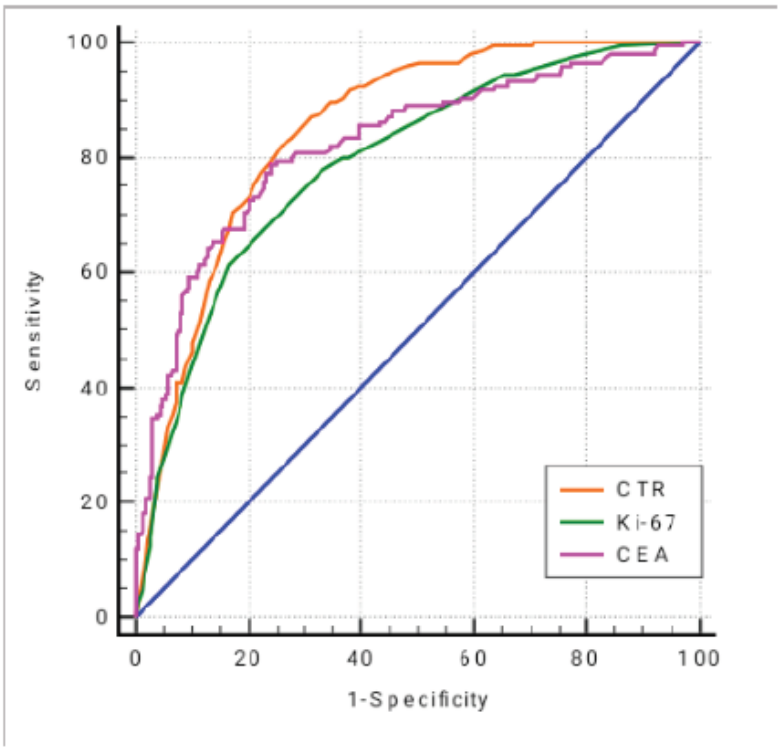

Figure 2

ROC curves of CTR, CEA, and Ki-67 index prediction probability constructed using MedCalc software to compare differences of three risk factors predicting pN

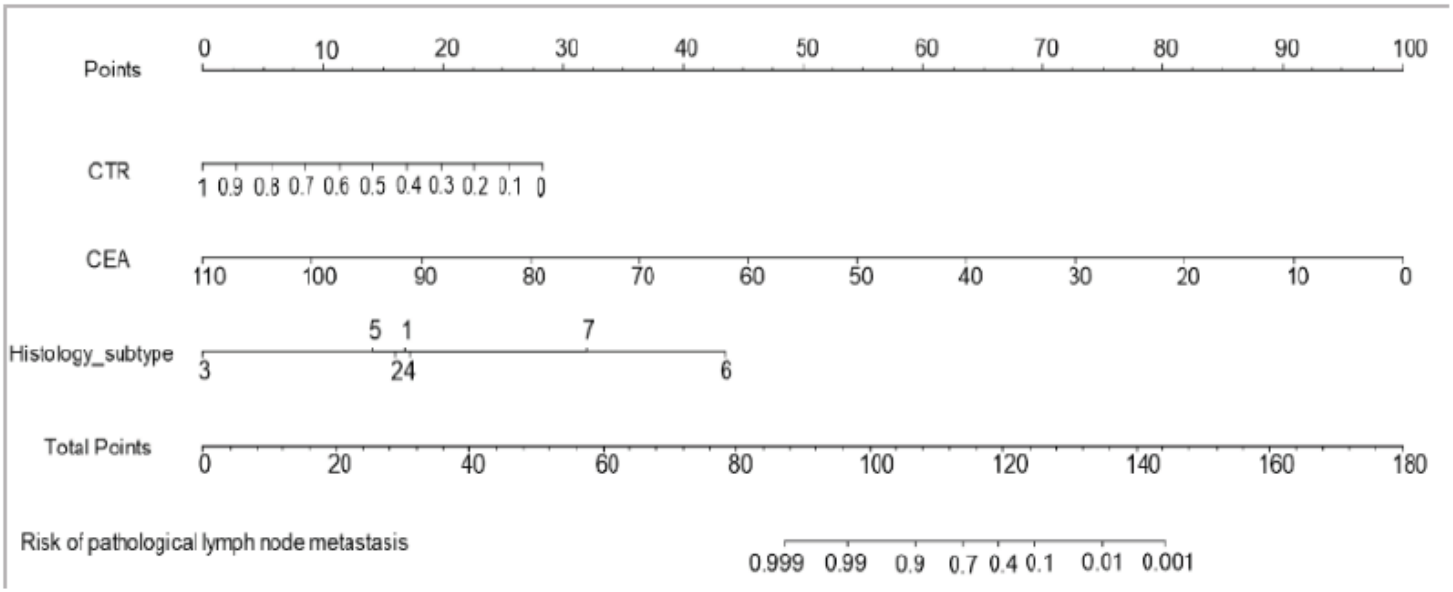

Figure 3

A Nomogram prediction model of pN established based on the CTR,CEA and Histology subtypes of the patients. Each score was added to get the total score and the pN risk

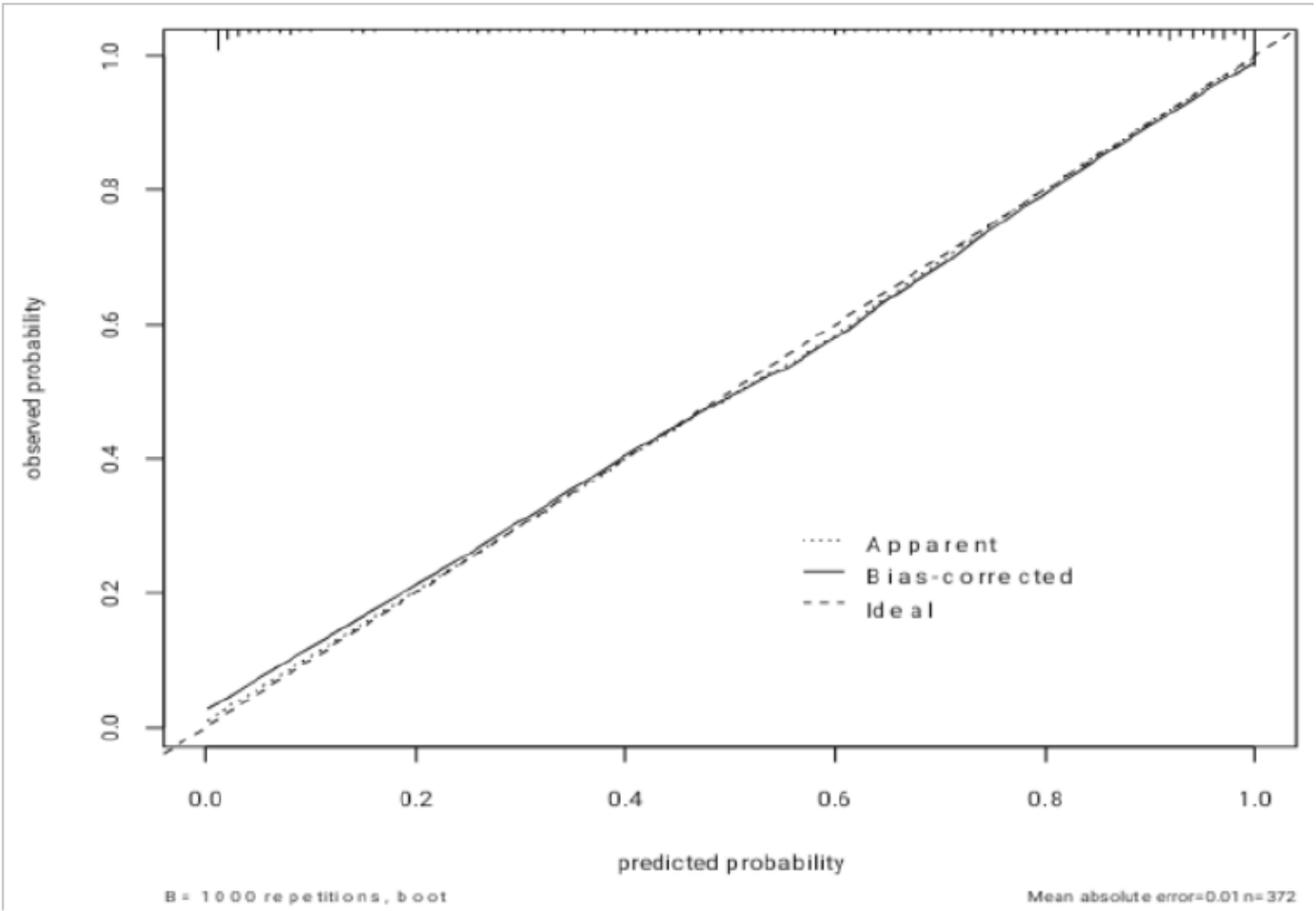

Figure 4

Calibration curve of the Nomogram prediction model